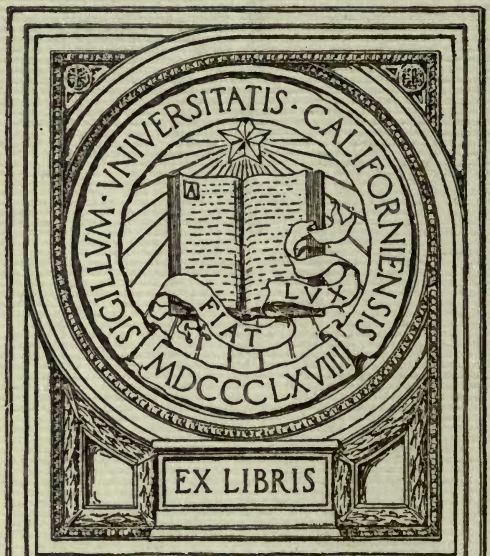

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Vol. XIV. BULLETIN Article V.

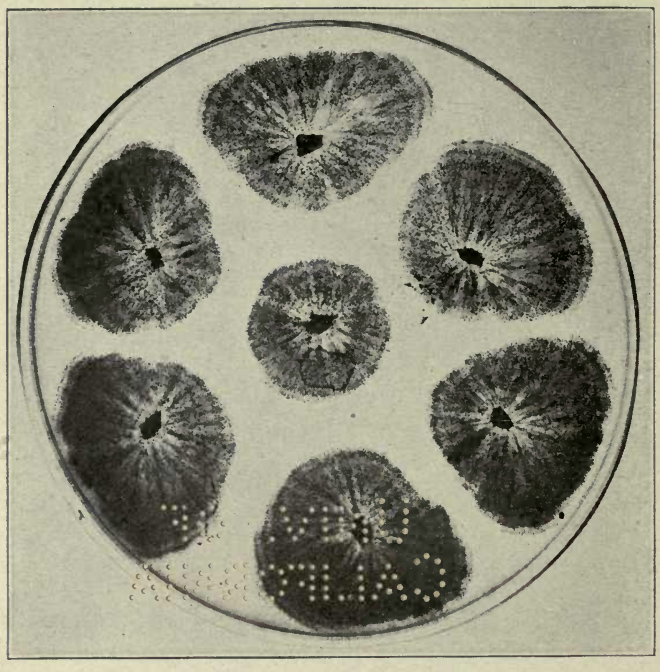
The Helminthosporium Foot-rot of Wheat, with Observations on the Morphology of Helminthosporium and on the Occurrence of Saltation in the Genus

BY

F. L. STEVENS

PRINTED BY AUTHORITY OF THE STATE OF ILLINOIS

URBANA, ILLINOIS
June, 1922



Original isolations of the foot-rot Helminthosporium made in May, 1919, from bits of tissue from wheat grown in Madison county, Illinois.

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CONTENTS

ARTICLE V.—*The Helminthosporium Foot-rot of Wheat, with Observations on the Morphology of Helminthosporium and on the Occurrence of Saltation in the Genus.* By F. L. STEVENS.

INTRODUCTORY

The present study of wheat disease is based upon a foot-rot, or rot of the basal portion of the stems, of wheat plants, as it occurred in Madison county, Illinois, in 1919 and subsequently. This disease was first reported in United States Government publications as "take-all" (*Ophiobolus graminis*); later, merely as "take-all," no cause being assigned; and for some time past, in Government publications it has usually been designated as "so-called take-all." An annotated bibliography of nearly one hundred titles concerning foot-rot disease of wheat, prepared by the writer, was presented before the Cereal Pathologists of America at St. Louis in June, 1919, and this, expanded to one hundred and eighty-eight titles, was published in October, 1919 (116). As early as May, 1919, cultural studies quite clearly pointed to Helminthosporium as the true cause of the disease, and at the December meeting of the American Phytopathological Society I announced this fungus as the probable cause. In May, 1920, in a note in *Science* (117), I published the statement that it had been conclusively established that this foot-rot of wheat is caused by Helminthosporium.

One purpose of the present paper is to present the evidence on which the foregoing conclusion is based and certain facts concerning the morphology and parasitism of the fungus; but far transcending in interest the disease itself—which now appears to be one of much less alarming nature than was at first feared—is the fact that very striking phenomena of variability are found in this and related fungi. In the following pages, therefore, appear (I) an account of the Illinois foot-rot of wheat and its causal fungus; and (II) evidence and discussion of the occurrence of saltation within the genus Helminthosporium.

ACKNOWLEDGMENTS

In this study I have been assisted financially by grants from the Illinois Natural History Survey and from the University of Illinois. I am indebted for specimens to persons mentioned in the list of species used for comparison (pages 181-184), and to W. P. Snyder for computation of data embodied in several of the graphs. I wish also to express my thanks to Prof. J. A. Detlefsen, who kindly read the manuscript and offered valuable suggestions regarding genetic questions.

I. A Foot-rot of Wheat and its Causal Fungus

Symptoms

As the name implies, the most obvious symptom is a rotting of the basal portion of the stem of the wheat plant, that is, the lowest portion of the lowest internode. In earlier stages than that of actual rotting, minute yellow or brown lesions occur on the stem (Pl. VII), while the roots, if diseased, are slightly yellowed and largely or quite devoid of functional root-hairs. No weft of superficial mycelium or black incrustation, such as is so frequently described in articles concerning take-all, was seen. The diseased tissues, however, were invariably ramified by an internal mycelium. Certain cases of diseased wheat came under observation in which the plants had attained a nearly normal growth and were eighteen inches high, when they suddenly died throughout. In such cases there was a slight darkening of the lower node and a mycelial invasion at this point. The opinion of those who observed this wheat in the field was that the death was due to frost injury. It is probable that the actual cause of death was foot-rot following the frost injury.

Fungi Present

Direct microscopic observation of the diseased tissues, in all cases of foot-rot examined, revealed the presence of an internal hyaline or faintly tinted mycelium in great abundance permeating the diseased tissues. Mycelium of different character was also occasionally found, but so inconstantly as apparently to have no actual relation to the disease. Isolations of the fungi present in the diseased tissues were made by two methods:

1. Direct planting of bits of diseased tissue on poured agar (corn-meal agar or wheat-straw agar). The diseased tissue was secured in as clean condition as possible by stripping back the enclosing sheaths, excising the diseased part with sterile tools, and tearing it apart in a sterile Petri-dish.

2. Direct planting of similar bits of diseased tissue after surface-sterilization in mercuric chloride (1-1000, 10 min.).

Dilution plating was unsatisfactory owing to the paucity of conidia and the presence of numerous soil bacteria, particularly "spreaders."

As might be expected, the methods employed gave rise to colonies of many genera and species, including Phyllosticta, Septoria, Fusarium, Epicoccum, Alternaria, and Helminthosporium. A striking fact, however,

was that with the exception of the Helminthosporium, these fungi were very rarely present, and then only a single colony or part of a mixed colony on occasional plates. Alternaria occurred with remarkable rarity; only two or three colonies among several thousand. Fusarium was found in only a few colonies and so mixed that it was isolated with difficulty. Epicoccum occurred in two colonies; Phyllosticta also in two colonies (two species).

A Helminthosporium, however, appeared in every plate and from nearly every bit of tissue used, no matter how great the care in securing the inoculum. On many plates this Helminthosporium (which throughout this article I designate as H. No. 1) appeared in pure culture Thus it may be said that the Helminthosporium was universally present in the plates; that it was the only organism that was present with any constancy; and that all other fungi were obviously strays.* Though conidia were never found in great numbers on plants brought in direct from infested fields, when the plants were placed in moist chamber for two or three days conidia developed in abundance. This was also the case with portions of wheat stems which had been placed in bichloride of mercury for ten minutes and then placed in moist chamber for several days. In passing it may be remarked that although great numbers of nematodes and amebae appeared in the plates there is no reason to believe that they had any relation to the disease under discussion or to any diseased condition.

Growth of the Causal Fungus on Various Media

Since the characters exhibited by various Helminthosporiums when growing in artificial culture have been considered as of importance as a means of distinguishing one species, variety, race, or strain from another, many media were employed in the present study. This was done in part for the purpose of comparing the growth characters of the Helminthosporium with characters reported by others in connection with other forms; in part with the hope that some of the media tested might give emphasis to certain characters and thus serve to differentiate between species or strains of the forms under observation.

The following notes are, in the main, statements of the characters presented by the foot-rot Helminthosporium (H. No. 1), though for the purpose of comparison notes are added regarding the growth of several

*A letter from Professor Hoffer written in May, 1919, tells me of a similar result from platings of wheat foot-rot from Indiana, and similar reports reach me from several other sources.

species or strains of Helminthosporium. These are throughout referred to by number rather than by name, partly for brevity and partly because the species of many of the races had not been determined, while in some cases the names were of more or less doubtful reliability. That the reader may formulate his own judgment of these forms, introduced for comparison, a complete list of them is given in the appendix (pages 181-184) together with certain notes regarding them.

VARIOUS AGARS AS MEDIA

Corn-meal agar in Petri dishes.—This medium, prepared after the directions given by Shear and Stevens (104), was found to be admirably suited to Helminthosporium and was the medium chiefly used.

The fungus grew rapidly, the colony being at first nearly hyaline both in the submerged and aerial parts, but when a diameter of about 2-3 cm. was attained the whole colony became much darker. Profusion of conidia was the chief factor in giving the dark hue to a colony, the slight darkening of the mycelium having little to do with it. The aerial mycelium varied largely with change of conditions, sometimes being very scant and at other times 5-6 mm. high, with windrow effects corresponding with the zones. After the colony was about 3 cm. in diameter zonation became quite pronounced, the zones corresponding approximately with the growth of each day. At room-temperature the colony attained a diameter of about 4.5 cm. in six days. Conidia-production was quite uniform over the surface of the colony unless checked by some growth-inhibiting cause, as drying, cold, or the antagonism of another colony near by, when it was much increased, as evidenced to the eye by black bands in such regions. By transmitted light the mycelium, and to some extent the conidia at certain ages, had a distinctly greenish tinge. H. No. 1 could be distinguished from H. Nos. 5-8, which were paler and produced fewer conidia. H. No. 6 approached nearer to H. No. 1 in these regards than did the others. H. Nos. 3, 4 (see Pl. IX), 6, 15-17, and 18 typically developed more aerial white mycelium than did H. Nos. 1 and 14. H. No. 36 was of very distinct character owing to large development of aerial mycelium (see Pl. X).

Corn-meal agar in Freudenreich flasks.—The flasks, of about 100 c.c. capacity, each received 50 c.c. of agar and were slanted. The large amount of nutriment available and the sustained moisture gave noteworthy characters. At 7 days, with H. No. 1, the surface of the slant was com-

pletely overgrown and of an even black color, largely curtained by an abundant, even overgrowth of white aerial mycelium. At contact with the glass a sharp, black line gave clear evidence of the black surface-coat. No clumps or balls of mycelium were present. At 22 days a few clumps developed, though not so many as on H. Nos. 9, 13-16.

Cultures of H. No. 1 on corn-meal agar in large flasks, as those of Kolle or of Piorkowski (Pl. XI-XIII) gave colonies very different from those on the ordinary Petri dish, due presumably to the larger quantity of nutrient available and to different humidity relations. These flasks gave increased density of colony and conidia-formation, more aerial mycelium, and some clumping of the mycelium. Though colonies of H. No. 3 and H. No. 1 differed in these characters in these flasks (Pl. XII, XIII), portions of the colonies of these strains were indistinguishable.

Corn-meal agar made at various temperatures.—Corn-meal agar was made in the usual manner excepting that the temperature in three cases was held at 43°, 85°, and 100° respectively, instead of at 60°, before filtering. Duplicate plates were made. The four resulting agars are designated according to the temperatures held, and colony data for each are presented in the following table.

Corn-meal Agar made at Various Temperatures

Temperature	Growth in 8 days	Zonation	Density	Colors
43° 43°	7.5 7.2	distinct	thin	pale
60° 60°	5.5 6	sharp	thick	dark
85°	6.5	In above characters, ranks between 43° and 60° agars		
100° 100°	8 7.8	none	very thin	very pale

The 100° agar is most favorable to linear growth, 43° agar stands next; 43° and 85° agars give growth of poorer color than 60° agar, but 100° agar ranks lowest in this regard. Color is directly due to quantity of conidia, and it is uniform in the mycelium on the four agars. Nutrition in 100° agar was very little better than in plain agar. In general, it

appears that their order of nutritive value for this fungus, from poorest to best, is 100°, 43°, 85°, 60°. Evidently a temperature of 43° is insufficient to extract the nutrient proteids sufficiently, while 100° precipitates too many of them. While leucosin, a prominent proteid of the embryo, is largely precipitated at 52° and a second coagulum goes down at 82°, no more is precipitated even by boiling (Osborne, 89).

Graphs 1-4 (Fig. A), indicating conidial length on these four agars, show that although the quantity of conidia produced varied materially, the length and general variability are not greatly influenced by varying the composition of the agar—done in this case by change of temperature. The conidial length of all these agars is, however, considerably less than that on wheat shoots (cf. graphs in Fig. A and Fig. K). Graphs of conidial breadth and septation on 43° and 60° agars given in Fig. B also show but little influence of these agars on these two characters. A "Difco" corn-meal agar, prepared according to my directions by the Digestive Ferments Company, gave growth-characters almost identical with those of my own 60° agar. On "Difco" beef-agar the conidia were short, and were frequently deformed (M, $17.44 \pm .22$, σ, $2.46 \pm .16$, CV, $14.15 \pm .93$).

Plain agar (shredded agar only, 12 g. per liter).—The fungus grew rapidly, and in 6 days the colony was 35–45 mm. in diameter, but was thin and colorless, with but few scattered conidia, and only 1 to 3, or at the most 7 to 10, conidiophores per low-power field, except at growth-inhibition points, as at the edge of the dish or where two colonies approached each other, where the number of conidiophores rose to about 12 per low-power field. The conidiophores bore only one or two conidia each. Conidiophores, conidia, and mycelium as well, were very faintly straw-colored, much paler than on more nutrient agar. No zonation occurred. No difference in rate or character of growth was observable in 1.3% and 2.6% plain agar.

Growth on plain agar, on corn-meal agars, and on various combinations of these nutrients.—Corn-meal agar of various compositions was used, 12 c.c. to each Petri dish. On 25% corn-meal agar (made of 3 parts plain agar plus 1 part ordinary corn-meal agar) the colony was much darker and denser than on plain agar; was zoned more strongly; and conidia were much more abundant, there being about 80 conidiophores per low-power field, each with one to five conidia. The mycelium was much darker than on plain agar. Colonies on 50% and 75% corn-meal agar showed no essential difference from the colony on 25% agar. On full corn-meal

agar the colony was much darker and more dense and the mycelium was darker. The relative rate of linear growth on these agars, as shown in millimeters of colony diameter at the end of 9 days at room-temperature, was as follows:

On plain agar.....70 mm.
On 25% corn-meal agar..68 mm.
On 50% corn-meal agar..62 mm.
On 75% corn-meal agar..57 mm.
On 100% corn-meal agar..54 mm.

When the fungus was planted on plain agar, and pieces (1 cm. square) of corn-meal agar of the above-mentioned compositions were laid on the surface at the edge of a well-developed colony, both color and conidia-production increased with the increase of nutrients. Variation in conidial length on these agars is shown in Fig. C. On this series of agars conidial length was least on plain agar and increased consistently with the strength of the medium. It is to be noted that the coefficient of variability is very high on the 75% corn-meal agar. In Graphs 9 and 10 of this Fig. C, conidial length is seen to be appreciably lower than on full corn-meal agar (Graphs 1-4, Fig. A), and markedly shorter than conidia grown under standard conditions (see Graphs, Fig. K; also App., page 180). Again, corn-meal agar was made in the usual way but the amount of agar was varied, 6, 12, and 25 grams per liter being used. In general, in Petri dishes, 12 grams per liter proved most suitable. Comparisons between H. No. 1 and H. No. 3 on these three media showed at 11 days each that H. No. 3 had grown more rapidly than H. No. 1, the ratio being 6.8: 8.5. There was usually a marked difference between these two strains on 12-gram corn-meal agar, more marked than on the others, H. No. 3 showing more definite zonation and more aerial mycelium.

In Freudenreich flasks, with the 6-gram agar, H. No. 3 made much aerial mycelium on the watery surface; H. No. 1 made only a black pellicle and no aerial mycelium. On 12-gram agar H. No. 1 made small growth of aerial mycelium and the colony surface was black, while H. No. 3 had much loose, woolly mycelium. At 11 days H. No. 3 on each agar had more aerial mycelium and more clumps than did H. No. 1. The most conspicuous difference was on 12-gram agar, while on 25-gram agar H. No. 1 had no clumps and H. No. 3 a few.

There is a clear, definite tendency in H. No. 3 to make more aerial mycelium and more clumps than H. No. 1, but this is so dependent on con-

ditions of moisture, in air and medium, that it is far from being a reliable character for separating the two.

Green-wheat agar.—(Formula as for corn-meal agar, substituting for the corn-meal live wheat leaves and stems which had been passed through a meat-grinder.) H. No. 1 grew well, producing a dense colony, but with weak zonation and with much woolly, white aerial mycelium, and but few and scattered conidia. This medium, while apparently very nutritious, favored abundant vegetation rather than sporulation, and was a poor medium for the differentiation of races.

To determine the effect of reducing the nutrients in green-wheat agar, this was combined, in varying proportions, with washed agar with varying results. On washed agar the growth of H. No. 1 was scant, colorless, and with no conidia, the colony diameter reaching only 5.5 cm. in 9 days. As the content of green wheat was increased, there was a gradual increase in density of colony and of aerial mycelium. In 9 days the colony diameters were as follows:

On 25% green-wheat agar..9 cm.
On 50% green-wheat agar..8 cm.
On 75% green-wheat agar..7.5 cm.
On 100% green-wheat agar..7.5 cm.

These colonies showed no dark color and only very weak zonation, and in the two high concentrations the aerial mycelium developed into a dense, closely felted mat. Conidia were produced scantily and varied greatly from the shape found elsewhere, being less tapering, more nearly cylindrical, and materially thicker (Graphs 13, 14, Fig. D). In some instances septation differed markedly—diminished (Graphs 15, 16, Fig. D). Many large conidia had no septa at all, and others had irregular or incomplete septa. It is evident that this medium, even at 25% strength, induces many abnormalities, and the very high coefficient of variability is especially striking. The differences in septation here noted were not constant on the same plate and were much more common at the drying edge. On this agar conidial length was less than under standard conditions (see appendix, p. 180).

Wheat-straw agar.—(Fifty grams of old wheat-straw, boiled 20 minutes and filtered.) Growth was poor.

"Difco" beef-agar.—H. No. 1 grew slowly but was very dense; surface even; little aerial mycelium.

Starch agar.—This medium proved to be of but slight differential value. The growth was of a dark color and of somewhat bluish tinge.

Bean agar.—H. Nos. 1, 3, 5, 13, 20, grew well on bean agar, all developing a dense, woolly, gray aerial mycelium. Zonation was poor, or obscured by the aerial mycelium. The five strains showed no differences in growth on this medium, which was therefore poor for differential use. In Freudenreich flasks there was a definite black surface-line and much tawny aerial mycelium in clumps.

Brazil-nut agar.—(Formula according to Spencer, 108.) H. No. 1 in test-tubes grew very rapidly and luxuriantly with small development of aerial mycelium and a distinct black basal line. The agar was rapidly cleared of proteid precipitate by the development of a proteolytic enzyme. In Petri dishes a thick, dense, woolly, snow-white aerial mycelium developed which entirely curtained the surface-blackening. The colony was surrounded by a broad translucent zone due to proteolytic action. This agar is valuable for the pure-white aerial mycelium that develops on it, and to demonstrate readily the proteolytic action, though it did not, even in these regards, prove to be differential, since all of fifteen strains tested upon it gave nearly identical responses.

Oat agar.—H. No. 1 at 10 days gave a distinct black surface-line and very heavy aerial gray growth. H. Nos. 1, 4, and 14 were indistinguishable on it.

Apple-fruit agar.—H. No. 1 gave a black basal line and abundant, sooty aerial mycelium. H. Nos. 1, 4, and 14 were alike except that No. 4 produced large sclerotia.

Apple-bark agar.—H. Nos. 1, 4, and 14 grew very slowly and were very dense and black, with but little aerial mycelium.

Czapec agar.—H. No. 1 gave a black surface-line and no aerial mycelium. H. Nos. 4 and 14 were of the same character except that No. 4 produced a considerable quantity of smoky aerial mycelium.

Prune agar.—H. Nos. 1 and 4 gave a dense, black surface-growth but no aerial mycelium.

SUMMARY CONCERNING GROWTH ON AGARS

Corn-meal agar made by the usual 60° formula proved most useful, and the best differential agar. If made at 100° or at 43° it lacked nutriment. The amount of agar used—even 25 g. per 1000 c.c.—had but little effect on growth characters. Green-wheat agar in varying strengths led to luxuriant vegetation, to little conidia-production, to much abnormality

in morphological characters, and was of little differential value. On either washed agar or plain agar there was excellent linear growth but poor color and little conidia-production. Bean agar gave too luxuriant vegetative growth. Brazil-nut agar was useful for the development of white mycelium for use in nutrition studies and for study of proteolytic action. The other agars used showed no special features of value.

RICE AND SIMILAR SUBSTANCES AS MEDIA, WITH SPECIAL NOTE OF COLOR PHENOMENA

Rice in test-tubes.—Rice was prepared in the customary way, by placing it in test-tubes to a depth of one centimeter, adding enough water to stand 1 cm. above it, and then autoclaving. This medium, so useful in the study of many fungi, notably of Fusarium, proved very interesting here. At the expiration of about two weeks from the time of inoculation—generally the most profitable time for first observation—three zones or regions could usually be recognized: (1) the region recently invaded by the fungus, which I designate as the *recent* region; (2) the region first invaded, which had assumed nearly its final appearance, and which I call the *old* region; and (3) the region midway between 1 and 2, which I shall call the *median* region.

Each of these regions showed characters of its own. Within all of them, but particularly in the old and in the median regions, there were three points to observe: (*a*) the places where the rice grains came in contact with the glass, which places I call *contact;* (*b*) the spaces between rice grains, at first filled by water, which I call the *interstices;* and (*c*) the line between interstices and contacts, which I term the *border.* Usually the fungus grows down into the interstices, consumes their contents, and fills the remaining space more or less completely with mycelium. Penetration of the contacts is very slow and may not occur at all, therefore they are usually but slowly discolored by the passage of various chemicals into them. The border is the region of greatest development, and often presents a sharp, distinct line of pronounced character. It is the contrasts furnished by the contacts, interstices, and borders, often attended by the development of beautiful and vivid colors, that give to these cultures their striking appearance. In addition to these characters the final appearance of the rice column should be noted. It is sometimes digested away in characteristic manner. The development or absence of sclerotia is also noteworthy.

H. No. 1, in rice test-tubes, at two weeks, gave, in the recent region, salmon-colored interstices, contacts, and borders; in the median regions the

interstices and contacts were gray, the borders olive; in the old regions the interstices were dark, contacts gray, and borders deep black. No sclerotia developed.

H. No. 2 gave quite distinctive color-characters, as did also Nos. 11, 12, 16, 17, and 19, including the colors pink, brown, red, gray, and purple. The remaining strains were distinguished only by sclerotial development, in which character strains known to be closely related differed markedly.

Additional notes made on these cultures at later periods, up to four weeks, differed only as to sclerotial development and the wasting away of the rice column. At four weeks H. No. 1 produced some sclerotia, and at five weeks, many.

In an attempt to ascertain wherein lay the responsibility for the various colors found in rice cultures, tubes were made in the usual way, using rice chaff, whole rice, rice from the pearling cone, rice from the brush, and rice polish. Of these, rice chaff and whole rice gave no color reactions; but all the others gave the usual ones. Single grains of autoclaved rice were placed on washed agar, under a cover-glass, and inoculated with color-producing Helminthosporiums. Direct microscopic observation showed the most intense colors on the unbroken rice-surface, and less intense ones on interior regions exposed by breaking. It is therefore probable that the proteids, which are most abundant in the surface layers, are necessary to the color responses, though the color itself often, if not always, arises from the mycelium.

Pearl-barley (prepared like rice tubes).—The general character of the growth as to contacts, interstices, and borders was as on rice but with different and less pronounced coloring. H. No. 1 at two weeks gave, in the recent region, salmon interstices, contacts, and borders; in the median region, gray interstices and contacts and olive borders; in old regions, dark interstices, gray contacts, and black borders; and the aerial mycelium was gray and the barley column not shrunken.

Navy beans (prepared like rice tubes).—H. No. 1 grown on this medium two weeks gave contacts unchanged, interstices gray to brown, borders brown to black. Little of differential value developed, though H. Nos. 11 and 19 gave pink colors.

Wheat grains (prepared as above).—H. No. 1 in old region gave contacts unchanged, interstices dark gray, borders black, and a very few sclerotia developed. H. No. 3 gave the same characters but more numerous sclerotia.

Almond integuments (*prepared as above*).—H. No. 1 gave a dense, black mycelium and abundant conidia.

Corn meal (*moistened and autoclaved in test-tubes*).—H. No. 1 gave dense, black borders and greenish-black interstices.

Corn-starch (*prepared as above*).—H. No. 1 gave a dense, black, even surface-growth with little or no aerial mycelium.

SUMMARY CONCERNING GROWTH ON RICE AND SIMILAR SUBSTANCES

With Helminthosporium, as with Fusarium, rice tubes are of value for differentiating species. The important constituent seems to be in the aleurone layer. Pearl-barley has similar and different, though less, value. Whole wheat grains, whole rice, beans, etc., do not give these color reactions.

Color phenomena in fungi have been discussed by Smith (106), and Hedgecock (65), and by Stewart and Hodgkiss (119), who cite several papers bearing upon the subject. These, however, deal mainly with conditions of acidity and general carbohydrates rather than with proteid relations.*

MISCELLANEOUS VEGETABLE MEDIA

Parts (plugs) of various vegetables were placed in test-tubes—in some cases with glass slips—with about 2 c.c. of water and autoclaved. The chief resulting characters of various Helminthosporium plantings on such media consisted in the development of aerial mycelium and the pellicle over the surface of the water.

Potato plugs (*prepared in the usual way*).—H. No. 1 gave on these plugs large development of woolly, white aerial mycelium. At the line of contact with the glass the growth was dense and black. No sclerotia developed in four days. This medium possessed little differential value, only H. Nos. 11, 12, and 17 showing clear differences.

Potato plugs on glass slips.—The slips were placed in test-tubes (in the manner shown in Fig. 2, page 95), and then slices of potato, which were so cut that they could barely be crowded into the tubes. They were then autoclaved. This gave opportunity for observation at three places: (1) the exposed potato *surface;* (2) the *contact* of the potato with the glass slip and with the wall of the tube; and (3) the *border* at the edge of the contact (cf. with terms under rice, p. 86). H. No. 1 here gave a dense black

*In this connection see paragraph on carbohydrates on page 100.

border, a woolly, smoky aerial surface, and was black at contacts. H. Nos. 11, 12, and 17 were otherwise colored.

Carrot plugs on glass slips (manipulated as above).—H. No. 1 gave black border and scant aerial mycelium; H. Nos. 11 and 17 were quite different.

On other carrot plugs, placed in test-tubes but without the glass slips, H. No. 1 gave at 4 days a slowly developing black surface-growth. H. No. 3 grew faster and with more aerial mycelium. At 9 days, H. Nos. 1 and 3 were alike.

Sweet-potato plugs.—H. No. 1 at 4 days was densely woolly on the surface; surface of H. No. 3, less woolly. At 9 days H. Nos. 1 and 3 were alike.

Onion bulbs; radish root.—H. No. 1 and H. No. 3 had abundant mycelium, a pellicle, and conidia.

Celery, onion stems, green peas, pea-pods, string-beans.—H. Nos. 1 and 3 had made dense, white, woolly aerial mycelium.

Rhubarb stems.—H. No. 1 made no growth; H. No. 3, poor growth.

Cranberries.—H. Nos. 1 and 3 made no growth.

Bean broth.—H. Nos. 1 and 3 gave a thick pellicle and a scant aerial mycelium. This medium has little differential value, though large white mycelial clumps appeared in H. Nos. 7, 8, and 9, much less in No. 6, and in some cases there was no pellicle. These characters, however, appear too variable to have value.

Old wheat-straw.—Old, dry wheat-straw, cut free of leaves and into lengths of about 12 cm., was placed in test-tubes with a few c.c. of water and autoclaved. Inoculated on these straws, H. No. 1 grew well, largely covering the lower part of the stem (which was in the most humid air) with a black coating of conidia. Each conidiophore usually produced several conidia. On the upper part of the stem, which was in less humid air, conidia were produced much less abundantly and were usually solitary on the conidiophore, while on the portion of the stem very near the cotton plug there was a growth of aerial mycelium. The fungus also grew sparsely over the surface of the water, where it produced conidia. Nineteen other numbers of Helminthosporium were grown on this medium and kept under observation more than three months. Three numbers, 11, 17, and 21, showed distinctive characters, and among the others there were minor differences in conidia production, in amount of aerial mycelium on the upper end of the straw, and in the density of the pellicle. These differences were, however, as great between two cultures known to be

of the same species (e. g., H. Nos. 5 and 6), and between H. No. 20 and No. 13, presumably of the same species, and therefore they are not of reliable taxonomic value. Even at the end of three months there were no sclerotia, pycnidia, or perithecia in any of the tubes. Ravn (91) has stressed the importance of sclerotia (which this medium yielded in his studies) as a means of distinguishing certain races or species; but no such value attaches to it for the races that I had under observation.

Fresh wheat-leaves.—Green wheat-leaves were prepared in the same manner as the wheat straw. On these leaves growth of H. No. 1 was much as on the wheat straw except that many more conidia were produced both on the lower portions of the leaf and on the water-surface. On the upper part of the leaf, about 6 cm. above the level of the water, where the leaf was too dry for conidia-formation, a rather extensive, white, floccose, loose aerial mycelium developed.

SUMMARY CONCERNING THE FOREGOING VEGETABLE MEDIA

But little of differential value resulted. The characters of aerial mycelium, sclerotia, clumping of mycelium, and pellicle-formation were but slightly marked, all being highly dependent on conditions of environment.

CEREAL SHOOTS GROWN FROM ASEPTIC SEEDS AS MEDIA

Cereal seeds were disinfected and sprouted in sterile moist chambers. When the shoots were 2-3 cm. long they were cut off, placed in water in a test-tube, and autoclaved. Next, washed agar was placed in Petri dishes and inoculated with H. No. 1. About four days later, when the colonies were several centimeters in diameter, various cereal shoots, prepared as above, were laid on the washed-agar plates, the basal end of the shoot in each case touching the edge of the colony. In this way repeated tests were made with wheat, oats, corn, rye, and barley, with the invariable result that growth was most luxuriant and dense on the corn, which soon became completely black. No constant difference was evident between the other cereals, except that rye seemed a medium slightly less favorable than the others.

It seemed probable that the more luxuriant growth and conidia-development on the corn shoots was due to quantity rather than to quality of nutrients. To test this hypothesis an entire corn shoot, a longitudinal half of a shoot, a longitudinal quarter of a shoot, and a mere longitudinal filament were laid on a colony of H. No. 1 growing on washed agar. On the smallest fragment, growth and conidia-production were about as on

wheat. The quantity of conidia produced, and correspondingly the black color, bore a direct relation to the mass of the corn shoot. It is probable that all of these autoclaved shoots are of equal, or nearly equal, nutritive value for a given amount of material.

Eleven other strains of Helminthosporium were tested in similar manner, using wheat, oat, and barley shoots. No significant differences in growth appeared, though, in most cases, at the end of the growth-period there was a somewhat more profuse development of conidia on wheat than on the two other cereals.

Environmental Factors which Induce Variation

Since classification of these fungi is necessarily based primarily on morphology, and on growth characters as exhibited on various media, it is important to know what, and how great, variations in these characters are induced by environmental changes. It is generally conceded that culture characters to be comparable must be noted under similar conditions. But how much latitude is permissible here; what difference in environment may be regarded as negligible? Fungi are collected that have grown under different conditions of nutrition, humidity, temperature, etc., and specific descriptions are written, based on these collections. To what extent are they trustworthy? The size and shape of the conidia and septation are regarded as of the highest taxonomic value. The development of sclerotia, of aerial mycelium, of mycelial clumps, of color, etc., are often the only characters separating some types regarded as species. Even the characters of the colony on the agar plate are considered important. It is with the hope of throwing some light upon this phase of the subject that I record the following additional observations concerning environmental factors which induce variation. More complete and exhaustive studies on these and kindred phenomena are needed.

Quantity of nutriment

Petri dishes were respectively supplied with 12 c.c. and 30 c.c. of 60° corn-meal agar and inoculated with H. No. 1. Placed under the same conditions, these dishes gave very different colony-characters. The colonies on the plates containing the larger amount of agar were very dense and black, and covered the agar-surface much more completely than did the colonies on the plates containing less agar (Pl. XIV). There was also marked difference in the rate of linear growth, as is shown in the following table:

GROWTH IN DIAMETER OF COLONIES (MILLIMETERS)

At 4 days' age		*At 11 days' age*	
On 12 c.c. of agar	30 c.c. of agar	On 12 c.c. of agar	30 c.c. of agar
pl. 1, 22	pl. 8, 32	pl. 1, 60	pl. 8, 85
pl. 2, 25	pl. 9, 40	pl. 2, 65	pl. 9, 90
pl. 3, 29	pl. 10, 32	pl. 3, 65	pl. 10, 85
pl. 4, 32	pl. 11, 40	pl. 4, 63	pl. 11, 85
pl. 5, 25	pl. 12, 35	pl. 5, 63	
pl. 6, 29		pl. 6, 62	
pl. 7, 27		pl. 7, 65	
Totals, 189	Totals, 179	Totals, 443	Totals, 345
Av. per pl., 27 mm.	Av. per pl., 35.8 mm.	Av. per pl., 63.3 mm.	Av. per pl., 86.2 mm.

It appears that at 4 days the colonies on the deep agar grew more than 32% faster than those on the plates with less agar; at 11 days, more than 28% faster, the colonies being at the same time more dense. A repetition of the test with 16 plates gave at 9 days a growth-average of 50 mm. on the shallow agar and of 74 mm. on the deep agar—an increase of 48% in rate.

Since it was deemed possible that the differences here noted might be due to differences in humidity, the depths of several plates were carefully measured and sufficient agar poured into each to make the air-space above the agar in all cases equal, although, since half of the plates were shallow and half of them deep, there was a great difference in the depth of agar used, which averaged in the deep plates about 11 mm. and in the shallow plates about 3 mm. The results of this test were almost identical with those recorded above. The differences noted, therefore, could hardly have been due to differences in humidity, but rather to the amount of nutriment available. The graphs of conidial length given in Fig. E show a larger predominance of short conidia on the deep agar, indicating the continuance of conidia-formation for a longer period of time. For this reason the mean length is much lowered and the coefficient of variability on the deeper agars is much higher than on the plates with less agar.

These results on deep agar are in entire agreement with those already given concerning corn shoots (page 90), to the effect that quantity of nutriment available may influence the growth-characters very markedly, often as distinctly as does the quality of the food available. The dilution experiments with green-wheat agar and corn-meal agar (pages 83 and 84) tend toward the same conclusion, though other factors also appear in those cases.

INHIBITORY INFLUENCES

Many influences which inhibit or retard vegetative growth, in so doing call forth increased sporulation, in accordance with the second law of Klebs (74) that the conditions favoring sporulation are always more or less unfavorable for growth. Thus, two colonies of H. No. 1 on washed agar were independently nearly devoid of conidia; but when they grew and approached each other, vegetative growth was retarded, and eventually inhibited, and each colony became dark in the region of inhibition, owing to much-increased sporulation (Fig. 1). Colonies of many other species of fungi affected H. No. 1 similarly under like circumstances, as did also drying out of the agar at the colony's edge. Similar changes in growth, and consequently in colony-character, occur on almost any medium, prominently on corn-meal agar, and they must be understood and reckoned with if colony characters are to be used for descriptive purposes.

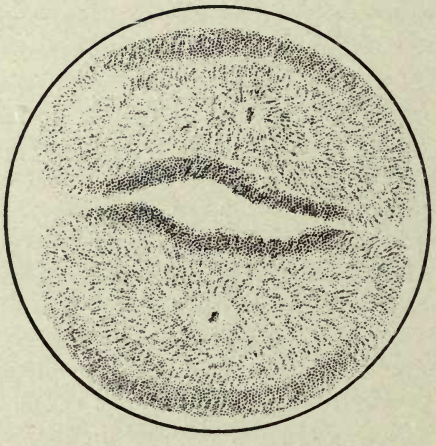


Fig. 1.—Illustrating inhibitory influence on sporulation. Two colonies of H. No. 1, on washed agar, showing dark bands, due to abundant sporulation where the colonies approach each other. Sporulation also somewhat increased near margins of colonies, owing to drying.

HUMIDITY OF MEDIA

Rice was placed in tubes with water equal to twice the volume of the rice, and with water equal to four times its volume, being then autoclaved and inoculated with various Helminthosporiums. Notes at two weeks show better characters of border, interstices, etc., in the wetter tubes, and while with many strains sclerotia developed abundantly in the drier tubes

they did not appear at all in the wetter ones (Pl. XV). This was notably true of H. Nos. 1 and 5. Even at the end of five weeks no sclerotia were produced in the wet tube by H. No. 1. It is apparent that the smaller amount of water is the more favorable for observation of sclerotium-formation, either at two or four weeks, and that observation of wet tubes at the end of two weeks is sufficient for other characters. Ravn (91) has suggested sclerotial formation as a character for the separation of certain species. It is obvious that if this character is employed care must be given to the humidity of the media.

HUMIDITY OF AIR

Test-tubes were prepared with water in the bottom and a glass slip inserted as is shown in Fig. 2, and autoclaved. If filter-paper or a wheat leaf is laid on the glass slip the humidity is sustained throughout the length of the tube by evaporation from the leaf or the paper, but if no such conductor of water is used, and cereal (e.g. wheat) shoots are laid crosswise on the glass slip (as indicated in Fig. 2) and inoculated, the growth of the fungus may be observed under different conditions of air-humidity.

Determinations kindly made for me by Dr. G. L. Peltier with especially accurate apparatus devised by Dr. C. F. Hottes, showed that moist wheat sprouts became shriveled and apparently dry in 3 hours at a relative humidity of 0 to 60%; in 12 hours, at 70% and 80%; in 24 hours at 90%, and that only in a relative humidity greater than 90% did the sprouts remain apparently moist for a longer time.

Test 1. Moist sterile wheat-shoots were placed on the glass slip (as shown in Fig. 2) with centimeter intervals between them, and inoculated with Helminthosporium No. 1. All shoots 3 cm. above the water-level dried within 24 hours, indicating that a relative humidity as high as 90% existed only in the region of the lowest shoot and that next above it. In the region of approximately 90% relative humidity many of the conidiophores became abnormally long (600 μ), and the basal part was of mycelial rather than of conidiophore character (cf. Fig. 3). It was apparent from observations on shoots in these various humidities that when the air was too dry for conidia-production there was a considerable development of aerial mycelium, which accounts for the fact that frequently the basal portion of a vegetable stem (e. g., celery or wheat) may bear conidia, while the upper part bears a tuft of woolly aerial mycelium.

Test 2. Old, dry wheat-straw with water in test-tubes was autoclaved and inoculated with H. No. 1. In the humid bottom the conidia-clusters

were large, as was also each conidium. Toward the dry end the conidia-clusters and the conidia both became smaller. From graphs of conidial length (Fig. F) it is obvious that the mean length of the conidium is much

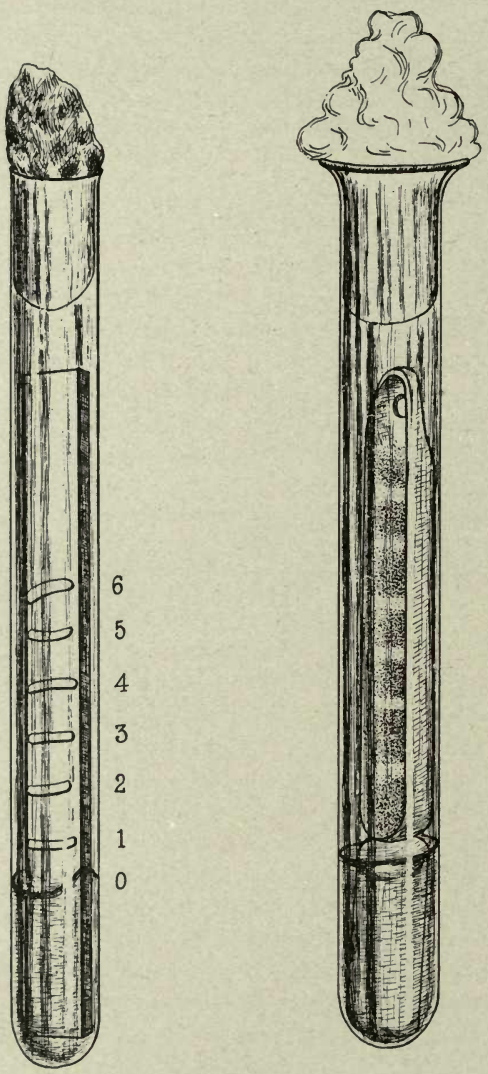


FIG. 2.—Showing at left, glass slip in test-tube: 0=water level, 1, 2, 3, etc., position of wheat shoots. In other test-tube, combustion boat supported on a bit of glass tubing, colonies growing on the agar in the boat.

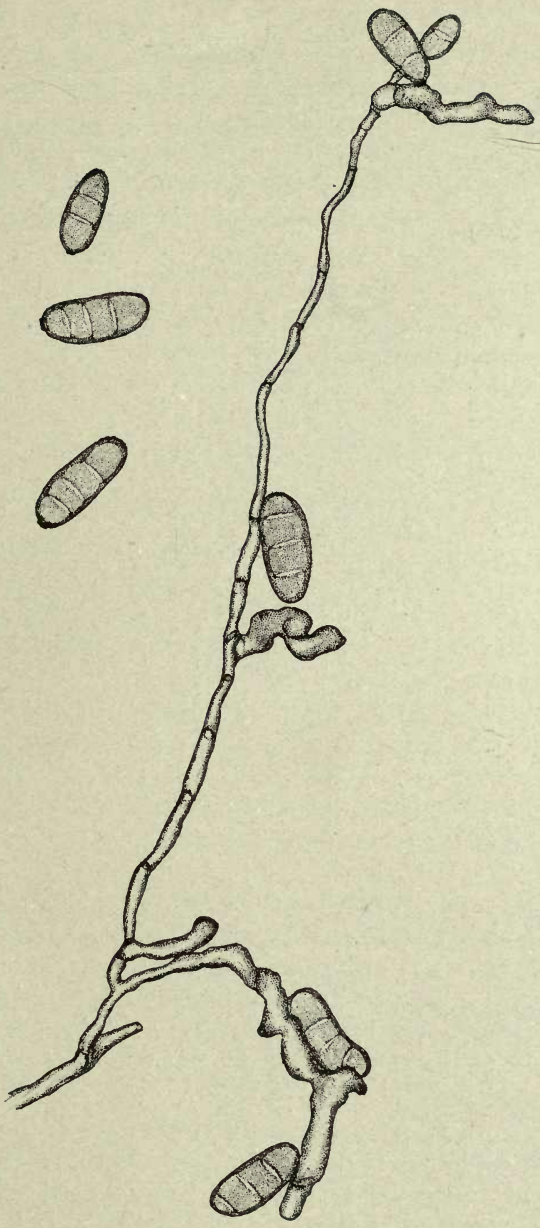


FIG. 3.—H. No. 21, showing origin of conidiophores from mycelium under humid conditions, the conidiophores being very short, thick, crooked, and black.

reduced by an environment of relatively dry air; also that the relative variability is much increased by these conditions (cf. data of Fig. F with data of Fig. A and Fig. G). The great influence of humidity on the morphology of conidia and conidiophores is discussed by Wartenweiler (124). Pammel, King, and Bakke show by respective illustrations (90, Pl. I, figs. 1, 2, 3, and 13-16) large variation in shape and size, and apparently in mode, of conidia of Helminthosporium as grown in the field and in the greenhouse. Similar effects from humidity were noted by Beach (12) in Septoria. The relative paucity of sporulation and the tendency of H. No. 1 to turn to the production of aerial mycelium unless the air-humidity was very high, and to proceed to profuse conidia-formation only when the relative humidity was above 90%, explains the failure to find Helminthosporium conidia on diseased wheat-stems in ordinary moist-chamber conditions, though these same stems, when given very moist conditions, invariably became covered with Helminthosporium conidia.

The six strains of Helminthosporium subjected to the humidity tests all agreed in essential behavior and characters with the reaction of H. No. 1 to the same, as given above; but there is apparent disagreement here with the conclusions of Ravn (91) who says that he placed "no water in the damp room (Boettcher chamber) since the moisture would be so great that it would prevent the development of conidia."

Test 3. Under a bell jar lined with filter-paper dipping in water—thus securing a close approximation to a saturated atmosphere—open Petri-dishes of inoculated corn-meal agar were placed. Under these conditions H. No. 3 made much more white aerial mycelium than did H. No. 1, and also grew faster, whether on thin (30 c.c.) or thick (60 c.c.) layers of agar.

It here appears that a condition of excessive humidity best develops the differential characters of the aerial mycelium of these closely related races.

Test 4. H. No. 1 was grown on corn-meal agar, and when the colony was well developed it was dried slowly until growth completely stopped. This resulted in a dense black band of conidia near the edge of the colony. There were many conidia upon each conidiophore, as many as thirteen being counted in one instance, and the conidia clusters resembled bunches of grapes. Only the oldest conidia of a cluster were at all large and these fell far below the mean as grown under standard conditions, while the other conidia were much below the usual size (cf. Graphs 62-64 [Fig. O] and 39-42 [Fig. K]) and extremely variable, with coefficient of variability 29.99 as compared with 12.22 under standard conditions (Graph 42, Fig. K). It

is obvious that such drying as is here recorded results in increase in the number of conidia per conidiophore; in great reduction in the modal mean and length; in increase in variability; and even in bimodality of length-graph. A count of scars per conidiophore gives the following: 1 case with 4 scars; 1 with 5; 3 cases with 6 scars; 3 with 7; 2 with 8; 2 with 9; and 1 case with 10. This record affords a marked contrast with the more usual condition of only 1, 2, or 3 conidia per conidiophore.

TEMPERATURE RELATIONS

The following is the record of H. No. 1 on 10°, 15°, 25°, and 30° washed agar at 87 hours: at 10°, trace of growth; at 15°, colony 10 mm. in diameter; at 25°, 21 mm. in diameter, at 30°, 19 mm.

GROWTH OF H. NO. 1 ON CORN-MEAL AGAR

Temperature	Dish No.	Growth of colony-diameter (millimeters) at time-periods indicated									
		2 da.	3 da.	4 da.	5 da.	6 da.	7 da.	9 da.	13 da.	17 da.	20 da.
10°	1	4	11	15	.....	18	22	29	40	49	55
	2	7	12	.....	.....	16	20	27	39	48	56
Average	.....	5.5	11.5	.....	.....	17	21	28	39.5	48.5	55.5
15°	1	8	18	28	39	50	61	76			
	2	10	21	31	39	48	55	67			
Average	.....	9	19.5	29.5	39	49	58	71.5			
20°	1	16	28	41	53	63	73	92			
	2	13	27	39	51	60	69	87			
Average	.....	14.5	27.5	40	52	61.5	71	89.5			
25°	1	17	34	48	61	75	90				
	2	19	34	48	60	74	90				
Average	.....	18	34	48	60.5	74.5	90				
30°	1	18	32	45	57	71	84				
	2	20	34	46	57	72	85				
Average	.....	19	33	45.5	57	71.2	84.5				

Steady increase in growth-rate is apparent up to 25° with a slight decrease at 30°. Bakke (6) places the optimum for *H. teres* as 23°—25°. Various changes in the appearance of the colony resulted from certain temperatures. Thus at 10° there was no zonation, no aerial mycelium, and very few conidia. At 15°, zonation was very faint, barely perceptible; at 20° and 25° more marked; while at 30° the aerial mycelium was very scant and zonation lessened.

Ravn (91) places the upper limit for growth at 33°—34°; the optimum at 25°—30°; the lower limit at 3°—5°.

Plates prepared in a similar manner to the above were kept for 4 days at 15° and then transferred to the 25° case. Similarly, transfers were made from the 25° to the 15° case. The data of colony-growth from this trial are shown in the following table.

GROWTH OF H. No. 1 IN ALTERNATING TEMPERATURES

Temperature	2 da.	3 da.	4 da.	Change of temperature to	5 da.	6 da.	7 da.	9 da.	13 da.
15°	8	18	31	25°	44	58	72	90	
	9	19	28		39	55	69	90	
Average.....	8.5	18.5	29.5	.....	41.5	56.5	70.5	90	
25°	17	32	48	15°	55	64	69	77	90
	18	31	47		54	63	71	78	90
Average.....	17.5	31.5	47.5	.....	54.5	63.5	70	77.5	90

The transfer to different temperatures made no perceptible difference in colony-character other than differences dependent on the rate of growth.

To ascertain the relation of temperature to the growth of H. No. 1 on live wheat shoots, this organism was planted as under standard conditions (App., page 180), except, of course, that the shoots were not autoclaved. Three temperatures, 15°, 20°, and 30° were used. When growth had proceeded to completion conidial length was found to be as shown in graphs of Fig. G. Conidia-production was scant at 30°, permitting only a few measurements. At the other temperatures it appeared to be normal. On these live shoots there was a marked shifting from the mean to the lower length as we passed from 15° to 20°, and a still more marked shifting at 30° (see Fig. G).

LIGHT

Two Petri dishes of corn-meal agar were inoculated with H. No. 1 and kept in the dark at 25°. In the following table the resulting growth is compared with that of similar plates kept in the light.

In darkness there was slightly less zonation and less aerial mycelium than in the light, but the difference was only slight and is probably insignificant.

To ascertain whether plate-position is an environmental factor of importance, numerous corn-meal agar plates were placed top up, others

bottom up, and observed carefully, but no difference in rates or characters of growth was observed.

GROWTH OF H. NO. 1 IN THE DARK AND IN THE LIGHT

Temperature	Condition	Growth of colony-diameter (millimeters) at time-periods indicated					
		2 da.	3 da.	4 da.	5 da.	6 da.	7 da.
25°	Dark	21	32	44	56	71	85
		21	33	46	59	74	85
Average		21	32.5	45	57.5	72.5	85
25°	Light	17	34	48	61	75	90
		19	34	48	60	74	90
Average		18	34	48	60.5	74.5	90

CARBOHYDRATES

Brazil-nut agar in Petri dish with H. No. 1 gave snowy white colonies. When such colonies had reached a diameter of about 3 cm. one oese of powdered dextrin, maltose, rhamnose, or glucose was placed on the agar a few millimeters from the advancing edge of the colony. Within 48 hours the portion of the colony near the added carbohydrate, with the exception of rhamnose, produced conidia in much greater abundance than before, and the mycelium turned slightly dark. Starch, corn-oil, and corn-meal produced a similar effect, but with delay of nearly 48 hours, suggesting that the additional time required was needed for the production and action of enzymes, diastase, or lypase, as the case might be.

Again, plain-agar plates were poured and inoculated with H. No. 1, and when the colony was well developed various carbohydrate nutrients were laid on the agar near the advancing edge of the colony: steamed rice, steamed tapioca, 1 square centimeter of standard corn-meal agar, a fragment of a Brazil-nut, corn-starch, wheat flour, corn-meal, and buckwheat flour. All of these nutrients were used, and in each case the colony in the region of these nutrients turned black, owing to the quantity of conidia produced (Pl. XVI). Ravn (91) noted a distinct relation between carbohydrate nutrients and blackness in Helminthosporium, as did I in various fungi (118).

On plain agar, glycocoll and aspartic acid inhibited strongly at first, but later the fungus grew through these. It grew normally through tyrosine, glutamic acid, leucin, cystine, phenyl, and alanine without percep-

tible influence on the number of conidia, though as it approached corn-meal agar conidia-production became profuse.

NUTRIENTS AS AFFECTING CONIDIAL LENGTH, SEPTATION, AND SHAPE

Plates of washed agar when solid were inoculated with H. No. 1, and when the colony had grown to a diameter of about 3 cm. one of various nutrients was laid on the agar in approximately equal volume, at a distance of 1 cm. from the edge of the colony. When growth had ceased, graphs of conidial length were made. These graphs, with data sufficiently explanatory, are given in Fig. H. While the number of measurements made is too small to warrant any definite conclusion as to nutritive values, the obvious general conclusion is that the added nutrient did markedly affect conidial length. It is particularly noticeable that washed agar plus saccharose, tapioca, or rice gave small conidia, and in none of these cases was modal conidial-length equal to that of conidia grown under standard conditions (see Fig. K). Even the very striking modification represented by the bimodal curve shown in Fig. I was a product of environmental change. It was noted in the sample from which the graph was plotted that the conidia were produced in rather large clusters, the oldest one being largest, the others mostly much smaller. The minor mode here apparently represents conidia in a stage of arrested development, comparable with those of Graph 62 (Fig. O), while the major mode stands for conidia that approached more nearly to normal development but did not attain full size (cf. graphs of Figs. I and O with those of Fig. K). That the bimodality is not due to

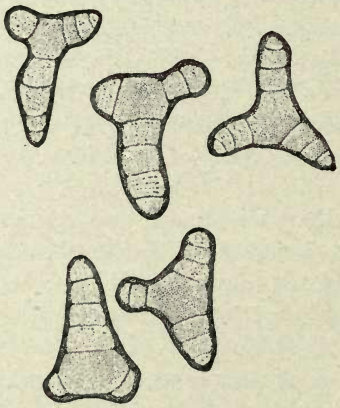


FIG. 4.—Tri-pointed conidia of H. No. 23 and H. No. 36 (see text, p. 102).

saltation is clear, since transfers made from these plates to wheat shoots (standard conditions; see App., p. 180) gave normal curves.

A striking temporary modification of conidial shape, due presumably to nutritive or osmotic conditions, was exhibited by H. Nos. 5, 23, and 36, in which a considerable number of the conidia were tri-pointed, owing to the central cell becoming inequilateral, then extending as is shown in Fig. 4. The fungus had long been cultured on rich carbohydrate agar, and when transferred to corn-meal agar these peculiar tri-pointed conidia were no longer common. (In this connection, see also page 154.)

SUMMARY CONCERNING ENVIRONMENTAL FACTORS WHICH INDUCE VARIATION

It is obvious from the foregoing record that very slight changes in environment may produce marked alterations in colony characters—growth-rate, color, aerial mycelium, clumping of mycelium, and even in the shape, septation, and size of the conidia. The quantity and concentration of nutrients, particularly of proteids and carbohydrates, have a very important influence on conidia-production and colony-color. Steinberg (114) and Javillier (69) have shown that even the zinc in the glass of the culture-flask has marked effect on the culture-characters. Dastur (40) has noted the following effect of media on Gloeosporium: after long culture on agar the conidia-bearing capacity is lost, though return to favorable media leads to its recovery unless the stay on agar has been too prolonged, in which case the capacity to bear conidia is completely lost. Air-humitidy is especially significant as regards aerial mycelium, and has also an appreciable influence on the size of the conidia. Light seems of less importance, though experiments were insufficient with this factor to be conclusive. Temperature in the upper and lower ranges has a marked influence on many morphological characters.

In the light of the foregoing experiments it is clear that comparisons of colony-characters on artificial media should be made (especially as regards points of minor difference) only on media known to be as nearly as possible of the same composition, and, when practicable, upon the same lot of medium and at the same time; thus only can truly parallel conditions be secured. Petri dishes with flat bottoms should be used, and in order to secure equal thickness of medium the agar should solidify when level. Straw, leaves, etc., in test-tubes, give different quantities of aerial mycelium and conidia of different size at different levels, both of which are due to humidity and

nutrition differences. It is clear that conidia developed in the open on leaves or stems—some of the conidia in humid conditions, some in dry; some on leaves of rich nutrient value, others on those less nutritious—may differ materially in measurement owing to environmental differences; and similarly, it is clear that sclerotium-development is markedly influenced by slight changes in humidity.

Ravn (91) states that conidia measured from one plant were thicker than those from another, but whether this was due to environment or to actual differences in the fungi was not stated. He does, however, state definitely that in his groups conidia collected under different conditions showed different lengths; and that their length depends upon the conditions under which they developed. Examples of extensive modifications due to substratum are mentioned by Edgerton (50), Coons (33), Duggar (45), Moreau (84), and Burger (32). Variations similar to those herein noted as due to substratum, especially those due to the relation of carbohydrate nutriment to color and alteration of conidial mode were noted by me in 1909 (Stevens and Hall, 118). Brierley (26) denominates such variation "modal variation."

Morphology of the Foot-rot Fungus

The parts chiefly to be considered, in the absence of knowledge of the ascigerous stage, are the mycelium, aerial, surface, and submerged, conidiophores, and conidia. Discussion of the relation of the mycelium in and to host-tissue will be given under the heading "Infection phenomena on wheat" (page 128).

This morphological study was made in part on the various media heretofore mentioned and on diseased plants in moist chamber. It was important, however, to have some means of securing the conidia, and other organs above specified, in large quantity, in form readily and conveniently available for examination. Moreover, the variability of the fungus under slight environmental changes emphasized the necessity of securing such morphological units as had developed under conditions as nearly identical and uniform as possible. This led to search for some standard means of culturing the conidia. Evidently corn-meal agar was unsatisfactory, since very slight changes in quality due to mode of preparation led to great morphological changes. Green-wheat agar and bean agar, both open to the same objection, also gave too much vegetative growth and too few and often abnormal conidia. Wheat in moist chamber, or wheat leaves or shoots in test-tubes varied so much in humidity that they could not give constant

morphological characters. The procedure finally adopted to secure standard conditions may be found in the appendix, page 180.

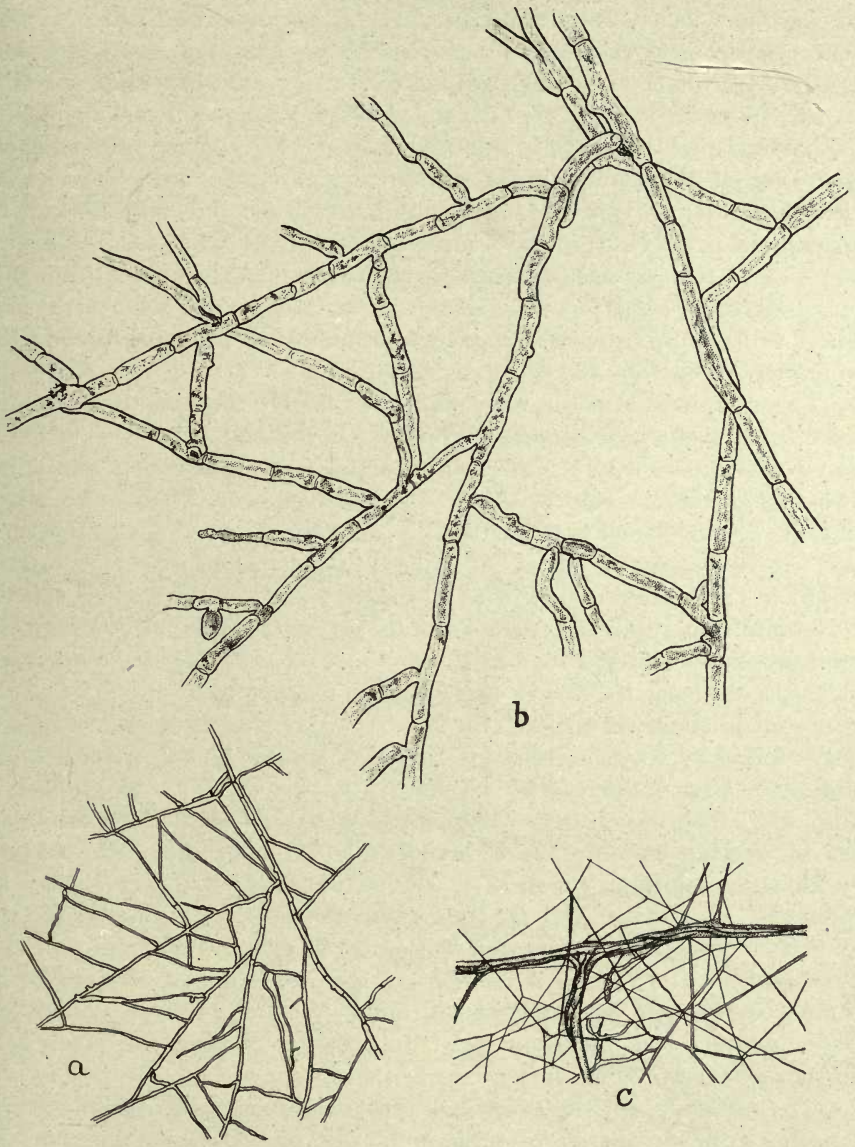


FIG. 5.—H. No. 1, showing extensive anastomosis of the mycelium where it coursed over bare glass: *a*, low-power view, *b*, detail of portion of *a*, high power; *c*, a bit of ropy mycelium composed of several twisted hyphae, many of the single threads undergoing dissolution.

MYCELIUM

The young, vigorous mycelium growing within the agar is smooth, quite straight, nearly or quite hyaline, and of very uniform diameter. The general aspect near the edge of a colony is shown in Fig. 6, *d*. In older parts of the colony the submerged mycelium is somewhat thicker and is less rich in protoplasm. The mature to old submerged mycelium has in agar a barely perceptible tinge of straw-color. The cells are quite strongly constricted at the septa and the content is highly vacuolate. Ravn (91) states that in the old immersed mycelium both plasma and cell-walls may be blackish green, grayish brown, or entirely black as in Alternaria. No such coloration as this has been observed in my cultures. The same author (91) discussing colony zonation, attributes it to variation in the color of the mycelium, while in my cultures it appears to be almost entirely due to variation in quantity of conidia and of aerial mycelium.

The quantity of aerial mycelium was highly dependent upon air-moisture and agar-conditions. When these favored there was a somewhat conspicuous, even floccose, white aerial mycelium 2 to 3 mm. high, consisting of either smooth, even, single filaments or, when quite abundant, of several filaments twined into a rope (Fig. 5, *c*). When conditions were less favorable the aerial mycelium, though inconspicuous, was still present in considerable quantity. The difference between various species of Helminthosporium and their saltants as regards the abundance of aerial mycelium is well shown in Plates IX-XIII. H. No. 36, a very distinct species from H. No. 1, was chiefly characterized by the great profusion of aerial mycelium (Pl. X). This character is the only one I have found by which to separate H. No. 1 and H. No. 3, and it serves only in the large cultures (Pl. IX-XIII), the distinction often failing in ordinary Petri-dish culture. The character is apparently so much modified by varying humidity as to make its utility for diagnosis of very questionable value (see pp. 95, 97). Ravn (91), however, distinguishes *H. avenae*, *H. gramineum*, and *H. teres*, growing on sterilized straw, by the different individual characters of the aerial mycelium and sclerotia; and *H. gramineum* and *H. avenae* on beerwort by the fact that *H. gramineum* forms a snow-white uniform mass of aerial hyphae, while *H. avenae* has sparse, white aerial mycelium which forms solid clumps, the black substratum-mycelium being visible between them.

The mycelium in wheat tissue is somewhat more irregular in contour than that grown in agar and is often much thicker. Occasionally upon the wheat surface it branched in a close fan-like fashion (Fig. 22, page 134). The

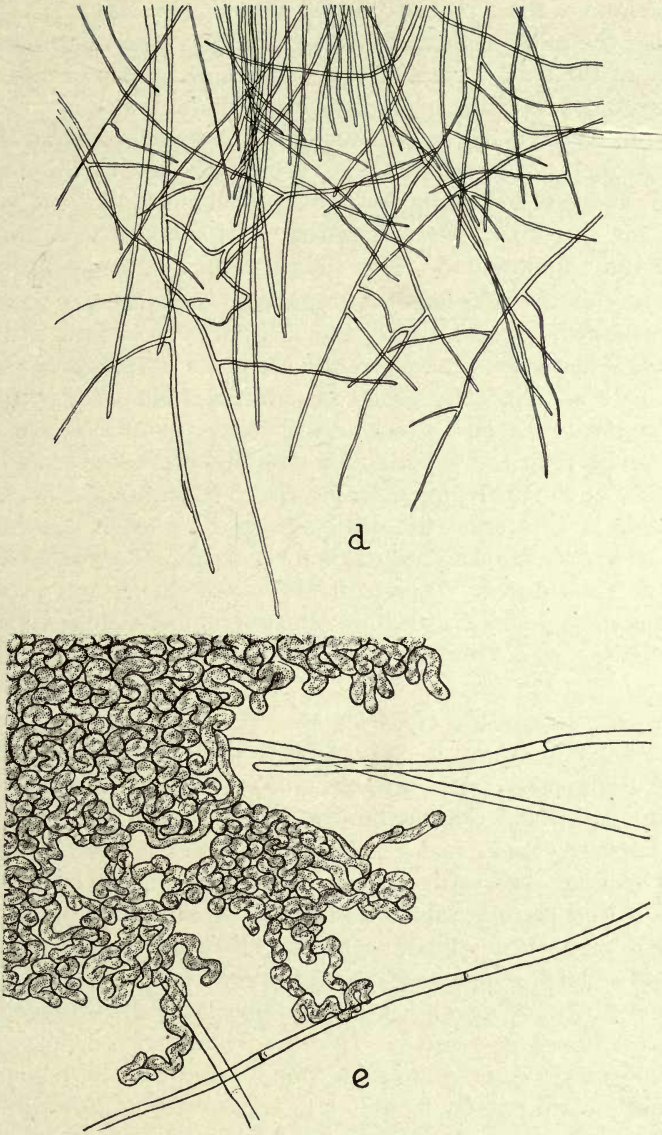


FIG. 6.—H. No. 1: *d*, showing branching and general appearance at edge of a colony growing on corn-meal agar; *e*, portion of a mycelial clump showing peculiar twisted character of the mycelium, with more usual strands for comparison.

aerial mycelium of some races produced clumps (Pl. XIII, XXII, XXIII), which under the microscope are seen to be due to a peculiar distortion and abundance of the aerial mycelial tips (Fig. 6, *d*). This peculiar behavior of the terminal parts of the mycelium shows some similarity to the branching figured by Ward (123) in Botrytis in the early stages of development of attachment organs. Anastomosis is very common with this fungus (Fig. 5, *a* and *b*), and Ward (123, fig. 19) has figured anastomosis of very similar character for Botrytis. Many citations of its occurrence are given by Beauverie and Guilliermond (13). (See also their figures 4 and 8.)

The nuclei in the mycelium are extremely small, but may be seen readily when stained with gentian violet, and still better if stained with iron-haematoxylin. They vary in number, but are never less than two and usually more; they do not typically group in pairs; and they are irregularly distributed in the protoplast. Nuclei apparently in mitosis are frequently seen, but since they are so small no details were noted except that the mitoses of all of the nuclei in one cell seem to be simultaneous, and mitosis was probable in adjacent cells. Reports on the nuclear conditions in the fungi imperfecti are few and unsatisfactory, doubtless owing to the extreme difficulty of the subject. Dangeard (39) notes nuclei and mitosis in the rather anomalous genus Bactridium; Beauverie and Guilliermond (13) in Botrytis; while Higgins (66) gives quite satisfactory figures for Mycosphaerella.

Senescence phenomena of aerial mycelium (Fig. 7, *a*—*b*).—When the aerial mycelium is young it constitutes a more or less abundant, loose, arachnoid, fluffy mass. In quite old cultures it is observed to mat down close to the surface of the medium in a thin, glazed, dead layer. Intermediate between these two extreme conditions interesting phenomena occur. The first observable change from that of the normal, vigorous mycelium is that certain cells of a filament, often many adjacent cells, become nearly or quite devoid of protoplasm (Fig. 7, *i*, *o*, and *g*), though cells at each end of such a series still appear normal (see *i* and *j*). Quite frequently the fungus re-grows from a protoplasmic cell, through the empty threads, as is shown in *n* and *o*. In other instances, and much more commonly, the empty cells gradually collapse until they remain as very thin, smooth filaments (*c*, *h*, and *m*), apparently of gelatinous texture. Where two filaments undergoing such dissolution cross they blend (*a*, *b*, *m*); and where several meet, rather large amorphous unorganized masses are seen, superficially much resembling a plasmodial structure (*a*). There was, indeed, at first, suspicion that there might be present a plasmodial

parasite preying upon the Helminthosporium mycelium; but numerous tests convinced me that such was not the case, but that what really occurs is that the old aerial mycelium dissolves (probably by auto-digestion). All stages of this disorganization can be followed under the immersion lens in stained preparations, where the disorganized filament stains with the gentian violet but is seen to be amorphous and without protoplasmic content. These phenomena appear to be limited to the aerial mycelium, but were

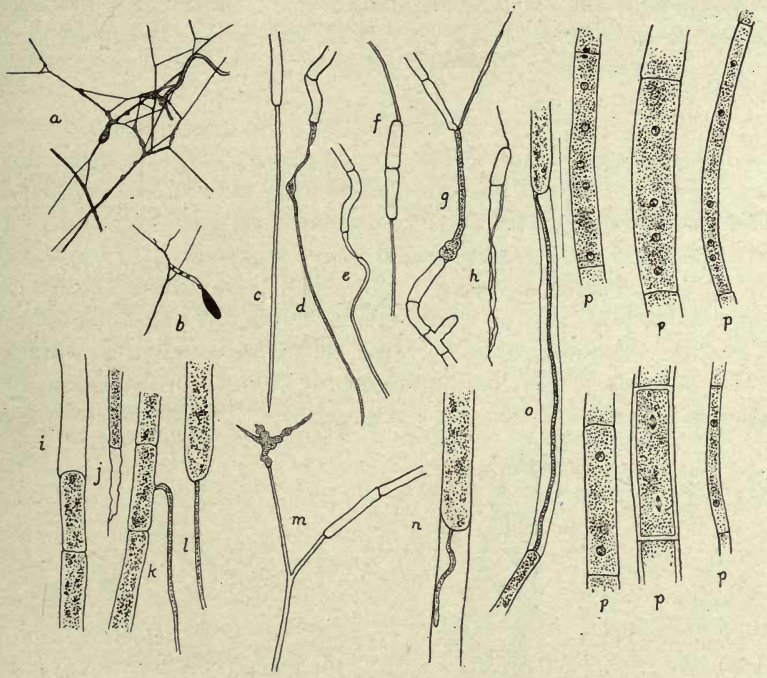


FIG. 7.—Various views (*a* and *b*, low power, *c*—*o*, high power) of mycelium of H. No. 1 in senescence: *a* and *b* showing dissolution to fine threads, *b* with a conidiophore still attached; *c*—*h* and *m*, empty mycelial cells adjacent to cells nearly dissolved; *i* and *j*, protoplasmic cells adjacent to empty cells; *k* and *l*, fine mycelial outgrowths from protoplasmic cells; *n* and *o*, fine mycelial threads growing from the protoplasmic cells and through old empty cells; *p*, bits of mycelium, as seen with the immersion lens, showing the nuclei.

observed on many strains of Helminthosporium. Autodigestion of mycelium doubtless occurs in the case of wood-rotting fungi, as is evidenced by the absence of mycelium where it was previously known to be, and it may be of common occurrence in other fungi. It certainly occurs when two hyphae join by anastomosis, and in the union of sexual organs. Growing-through of the mycelium, as noted above, and even conidia-formation within the old cell are common in Saprolegnia, and have been noted in

several other genera: Botrytis (Beauverie and Guilliermond, 13); Alternaria, Epicoccum, and Botrytis (Linder, 78); Inzengaea (Borzi, 23); Dematium, Botrytis, Oidium (Klöcker and Schiorming, 75); Chaetomium (Zopf, 129, figs. 24, 25, A, B, Tab. 16). The phenomenon, as described, is always associated with senescence. Sclerotia are described by Bakke (6) and by Noack (87), who seem to have found them common on old straw-cultures, varying in length from 200 to 600 μ. I have not found them at all on straw, though on old rice-cultures they are abundant. Pycnidia and pycnoconidia, as seen by Ravn (91) in *H. teres* and as described by Bakke (6), I have not seen.

CONIDIOPHORES

On standard wheat-shoots.—The conidiophores are in no sense clustered but arise singly as lateral branches, each from an ordinary mycelial cell, and differ from the mycelium chiefly in that they grow erect and straight instead of declined and crooked, and are darker in color than the mycelium. Usually this branch in its basal region is mycelium-like, but it rapidly thickens and darkens to true conidiophore character, and is usually 2.5 to 5 μ in length. Sometimes the mycelial cell from which the conidiophore arises also darkens. The conidiophore-cells contain protoplasm, and the protoplast plasmolizes under the usual reagents. When mature the conid-

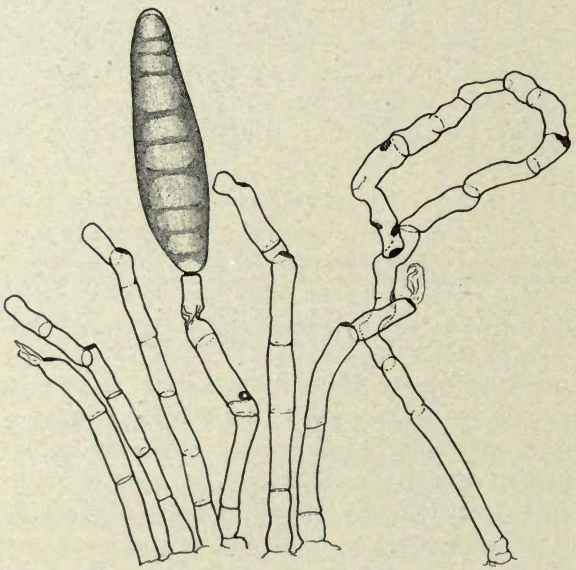


FIG. 8. — H. No. 1, showing variation in conidiophores, geniculation, conidia-scars, and septation.

iophores are pale straw-color to smoky brown from tip to, or nearly to, the base, the color being due to the outer wall—which is very brittle. Upon the production of the first conidium, which is strictly terminal, the conidiophore grows onward, with a slight bend where the first conidium was produced, and proceeds to bear another one. This may continue until many conidia have been borne by the same conidiophore. If the conidia are undisturbed, the cluster may have a botryose effect, but if disturbed, only the youngest conidia remain, and scars and geniculations mark the places of origin of the fallen conidia (Fig. 8). The number of conidia borne per conidiophore in count of 24 was as follows:

Frequency,	15, 5, 4
Conidia,	1, 2, 3

Much higher numbers than this occasionally occurred under standard conditions (see appendix, page 180); and much higher numbers were the rule on corn-meal agar.

The number of septa below the first scar varied from one to four, while the length in seven measurements from base to first scar was 78—88 μ. The length above the first scar is entirely dependent upon the number of conidia borne on a given conidiophore; in some cases it is equal to or even greater than the length below the first scar.

Conidia develop very rapidly upon the conidiophores. One of the latter kept constantly under observation was first observed at 11 o'clock to have a diameter of 6.8 μ; at 11:15, 13.6 μ; at 11:30, 20.4 μ; at 12, 37.4 μ; and at 12:30, 44.2 μ.

The conidiophores of certain other numbers, for example H. Nos. 2, 21, and 29, are of such very different character that the conidiophores alone serve to distinguish them markedly from H. No. 1. The conidiophores of H. Nos. 3, 5, 15, 16, and others, however, are closely like those of H. No. 1; indeed no real distinction could be found between them. Attempts were made to distinguish between these strains or species by plotting conidiophore length, septation, length of cells, etc., but nothing came of such attempts. The length of the conidiophore is markedly influenced by air-humidity (page 95), and it is probable that the rudimentary conidiophores may be changed into aerial mycelium by a lowering of the air-humidity.

CONIDIA

The conidia and their attachment to the conidiophores are shown in Plate XVII. From the basal end of the conidium to the conidiophore there is an exceedingly short (2×4 μ) black stipe. As the conidium falls

away from the conidiophore the stipe remains attached to the conidium, and as it can always be seen readily when the conidium is in suitable position, it serves as a ready means of recognizing the basal end of the conidium. The stipe is equally obvious and distinguishable in H. Nos. 3, 4, 5, 13-16, etc., though in H. No. 2 and certain other numbers the stipe is of somewhat different type. While in very rare instances catenulation of conidia was observed (Fig. 9, *b*), this is apparently much less frequent than in the forms

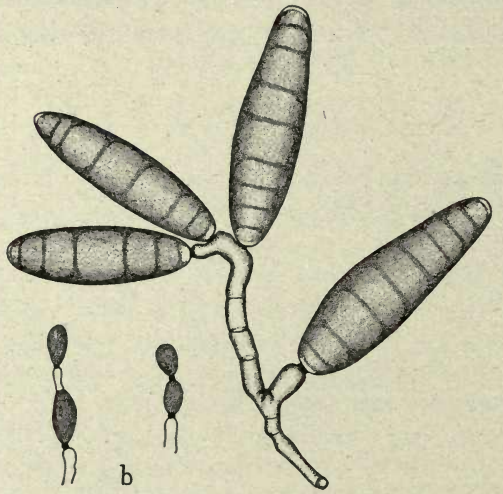


FIG. 9.—H. No. 1: *a*, portion of a conidiophore bearing conidia; *b*, catenulate conidia—rarely occurring.

described by Ravn. The apical end of the conidium is always obtuse, and is marked by a pale spot that was mentioned by Ravn (91) as occurring in *H. teres*, etc. Being of so distinctive a character, this end is always recognizable when the conidium is in a suitable position. We have, then, reliable means of identifying each end of the conidia: the basal stipe and the apical spot (Fig. 10). The latter, though not characteristic of all Helminthosporiums—for example, H. Nos. 2, 29, and others lack it—is characteristic of H. Nos. 1, 3, 13-16, and others.

The color of the conidia of H. No. 1 ranges from pale-straw to light brown, and under some conditions shows a slight bluish-green tinge. While H. Nos. 2 and 28 were distinctly and constantly different from H. No. 1 in color, H. Nos. 1, 3, 13-16, etc., were indistinguishable on a color basis.

The conidial outer wall.—This wall (the episporium of de Bary, 9), which gives the color to the conidium, is extremely thin and very fragile

(Pl. XVIII). It is so brittle that by gently tapping the cover-glass over conidia the outer dark wall of every one of them may be broken in fragments, much as a peanut is broken if stepped upon. This character is common to H. Nos. 1, 3, 5, 13-16, etc., as well as to H. No. 2, and many other species, though in some the wall is less brittle than in others. The conidial wall that is left after the solution of the endosporium by sulfuric acid is entirely without sign of septation, but shows the apical spot clearly differentiated as a thin pale region.

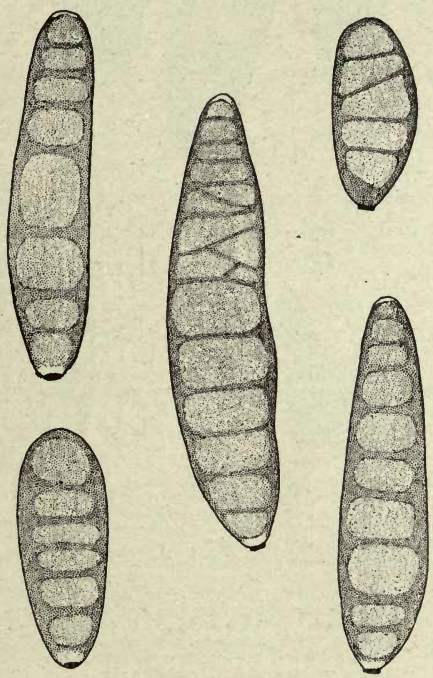


FIG. 10.—Variation in conidial shape and septation of H. No. 1, and showing also the dark spot, stipe, at basal end, and the pale apical spot.

Conidial contents.—Within the thin colored wall are the protoplasts, usually several in number (Fig. 11), and between the protoplasts and the outer wall is a thick hyaline layer of substance that is somewhat soft, usually appearing almost gelatinous (Pl. XVIII). This hyaline soft layer represents morphologically, I believe, a second cell-wall, the endosporium of de Bary (9). I shall so speak of it. That this wall is soft is shown by the way the conidial contents issue from the end of a cracked conidium under pressure

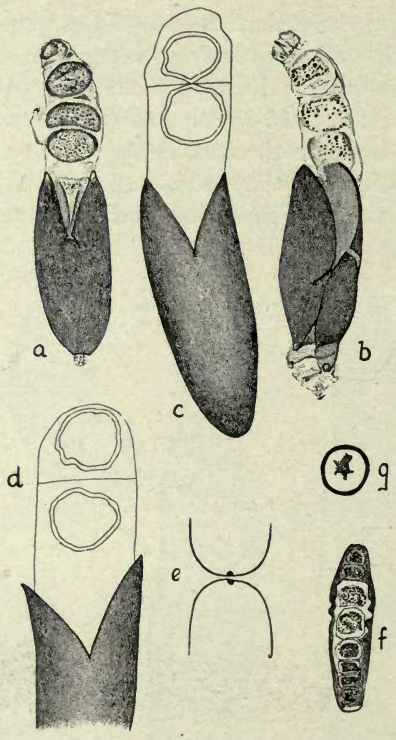


FIG. 11.—Conidia of H. No. 1: *a* and *b*, with outer wall cracked open by pressure, the inner hyaline wall and the protoplasts emerging; *c*, another conidium with the outer wall crushed by pressure, the two protoplasts walled and touching; *d*, similar to *c*, but with the protoplasts separate; *e*, immersion-lens view of two protoplasts within a conidium, showing thickening at their point of nearest approach to each other; *f*, a longitudinal microtome-section of a conidium from which both sides have been cut away; *g*, a cross-section of a conidium showing much clear space between the protoplast and the outer wall.

(Fig. 11). De Bary (9) remarks that the endosporium often shows great softness and delicacy but is by no means always thinner than the other wall. That the outer conidial wall has no internal ridges, and takes no part in forming septa, is shown both by direct observation and by inference from the way in which the conidial contents slide, unobstructed, lengthwise of, and out of, the outer conidial wall. Ravn (91) states that in the three species studied by him the walls and septa are very thin, but when treated with glycerine, etc., the outer wall becomes prominently thickened,

as also the cross walls where they meet the outer wall. In making this statement he refers to the episporium and endosporium as constituting two layers of one wall. In some instances the endosporium is clearly seen to extend between, and to separate, the protoplasts (Fig. 11, *b*), while in other cases the protoplasts appear to touch each other (Fig. 11, *a*), yet when the conidial contents are pushed from a crushed conidium there is always a line, though sometimes it is very thin, separating the protoplasts. Since the protoplasts are distinct from each other, and are thus separated by the endosporium, it seems justifiable to assume that this second cell-wall forms the septa, sometimes obvious though very thin, between the protoplasts. Treated with concentrated sulfuric acid the conidial endosporium dissolves rapidly, and by the generated pressure the episporium is ruptured, invariably at the basal end first, this often opening trap-door-like, though frequently the pressure is sufficient to tear the wall of the conidium open throughout its length. With the solution of the endosporium the protoplasts issue from the case of the conidium and appear to be unattached.

The individual protoplasts vary much in shape, sometimes being nearly spherical; in other cases nearly cubical. Each protoplast is surrounded by a differentiated layer which in some cases is so clear, distinct, and thick as to appear to be a third wall (Fig. 11). Perhaps it is. Under gentian violet and many other aniline stains, while the protoplast takes a strong stain this layer refuses to stain. Microtome cross-sections and longitudinal sections of conidia verify the foregoing conclusions (Fig. 11, *f*). In cross-sections (Fig. 11, *g*), with Fleming's triple stain the protoplast stains as usual, but the second cell-wall refuses to stain; under Bismarck brown it takes a very faint stain. Under action of aniline blue, iodine, fuchsin, malachite green, Pianese, or chlor-zinc-iodine it remains unstained. In longitudinal sections cut so thin that two sides of the conidium have been cut away, mature conidia show no continuity of the protoplasts (Fig. 11, *d*). When plasmolized the protoplasts all shrink and lie quite separate from each other, and it is in such condition that the appearance of a third conidial wall is most evident (Fig. 11, *a*, *b*). Previous to plasmolysis the protoplasts are frequently seen to touch each other on the median longitudinal axis of the conidium, and a very faint line (plane) is observable extending across the conidium (Fig. 11, *a*, *b*). This probably represents the true septum, following nuclear division. The protoplast wall bears a small dot-like thickening (Fig. 11, *e*) adjacent to its sister protoplast, which may also be residual evidence of nuclear mitosis.

The characters as here given for H. No. 1 are found also in such re-

lated forms as H. Nos. 3, 13, 16, etc. That the internal structure of Helminthosporium conidia has not been clearly understood is shown by numerous published figures.

Conidial germination.—The conidia germinate readily in water, in hanging drop, or on the surface of wheat shoots (Fig. 12), and germination,

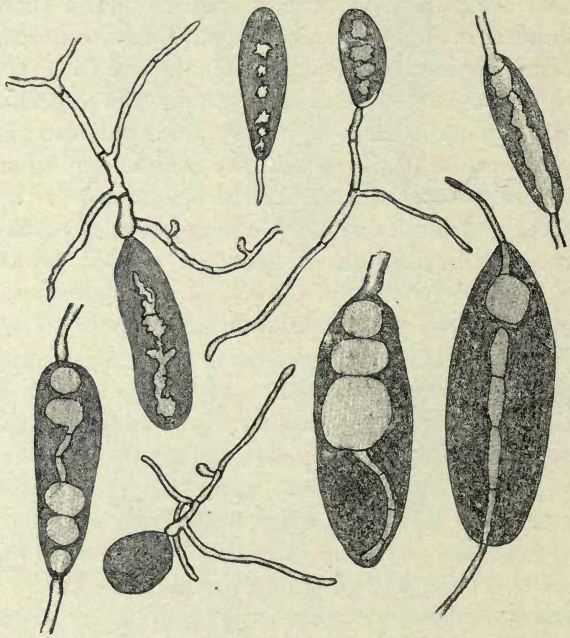


FIG. 12.—Germinating conidia of H. No. 1

so far as I have seen, is very rarely lateral but usually from the ends, most commonly from the basal end. Thus twenty-seven basal germinations were counted as against fourteen apical ones. The germ-tube is hyaline, richly filled with protoplasm, and forms abundant branches and septa (Fig. 12). Bakke (6) states that "germ tubes first come from basal and apical cells; later other germ tubes may arise from the remaining cells under favorable conditions." Kirchner (72) states that germination in *H. gramineum* is usually terminal, but Noack (87) shows that for this species the germ-tubes are as often lateral. The viability of the protoplast was not injured by crushing the epispore; indeed such cracking seemed to facilitate emergence of the germ-tube. Anastomosis of the germ-tubes is not uncommon (Fig. 13). As the germ-tube enlarges there is frequently, though not

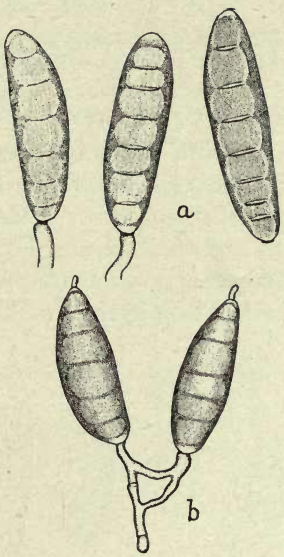


FIG. 13.—Conidia of H. No. 1: *a*, showing septa from two depths of focus; *b*, two germinating conidia with germ-tubes anastomosing.

always, shrinkage of the protoplasts such as is shown in Fig. 11, this shrinkage being usually most pronounced in the end of the conidium showing most vigorous growth. To all appearance the endosporium serves as a stored food and is consumed in germination, since its presence in much diminished quantity in germinated conidia is evident when such conidia are crushed. The conidiophore-cells also occasionally function as conidia by sending out a germ-tube. Here, too, the inner cell-wall seems to serve as reserve food.

Longevity of conidia.—It is not known how long conidia live, but on wheat straw that had remained air-dry for fourteen months they germinated normally. Noack (87) mentions germination "after many months." Ravn (91) says of three species that at eight months they germinated but sparingly or not at all.

Frequency of conidial septa.—H. No. 1 under standard conditions (see appendix, page 180) gave the graph in Fig. J, while similar data for H. Nos. 13-16 are given in Fig. T.

Septa	*Differences of means of septa*
H. Nos. 1 and 3	0.27 ± .16
H. Nos. 1 and 15	0.80 ± .12
H. Nos. 1 and 16	0.58 ± .11
H. Nos. 15 and 16	0.22 ± .11

It is to be noted that the differences between Nos. 15 and 16 are quite as large relative to the probable error as are the differences between Nos. 1 and 3. Ravn (91), speaking of three species of Helminthosporium, says that the septa are very variable, and that specific differences can not be derived from them. Very abnormal septation was frequent; for example, on green-wheat agar (Fig. 14) and other uncongenial media.

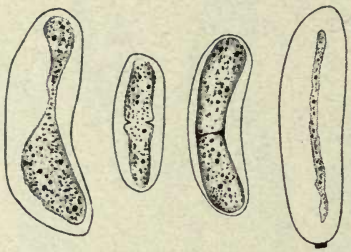


FIG. 14.—Various abnormal conidia of H. No. 1 as grown on green-wheat agar.

Conidial shape.—The shape of the conidia, together with their size and septation, are in the genus Helminthosporium the three most-used characters in description. Indeed in published descriptions of many species these are the only important characters mentioned, and often one or more of these is lacking. Conidial shape in certain species is very characteristic, particularly in *H. inaequalis*, *H. geniculatum*, *H. ravenelii*, and also in my H. No. 29. Much stress has been laid on conidial shape as a means of distinguishing certain cereal Helminthosporiums, particularly in distinguishing *H. sativum* from *H. teres*.

Merely to look at two lots of conidia with the microscope, even with the aid of a comparison ocular, is not a satisfactory means of ascertaining the prevailing conidial shape. Many strains of Helminthosporium vary greatly as to conidial shape, and conidia of one shape are mixed with those of another (Pl. XIX–XXI). The important question is, what is the relative frequency of the various shapes? But before any fair estimate of this can be made, standards must be established as to what are the essential characters of the various shapes.

One factor of preponderating influence in determining these conidial shapes is the position that the point of greatest diameter occupies on the

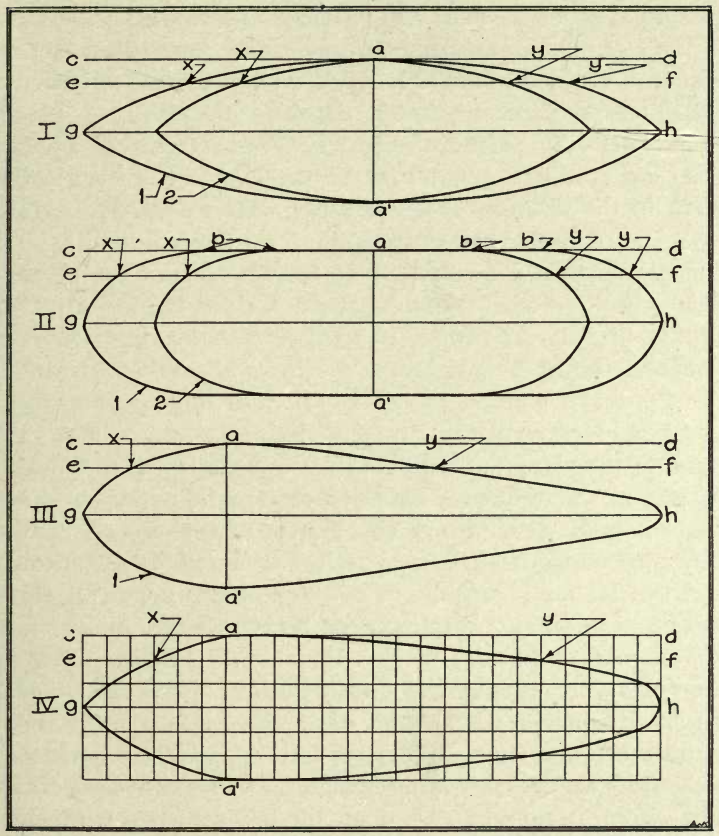


FIG. 15.—Diagrams elucidating conidial shape.

longitudinal axis of the conidium. In diagrams I and II, Fig. 15, the point of greatest diameter on the line *a*—*a'* is midway between the base and apex of the conidium; while in diagrams III and IV it is nearer to its base. If the conidium tapers from the point of greatest thickness toward each end a fusiform (Diagr. III) or elliptical (Diagr. I, 1, 2) conidium results. If for a sufficient distance on each side of the line *a*—*a'* the conidium remains of uniform diameter it approaches more nearly the form of a cylinder (Diagr. II, 1, 2). When the maximum diameter is nearer to the base than to the apex, somewhat rapid tapering gives a fusiform conidium (Diagr. III), but if (as in Diagr. IV) the diameter lessens very gradually, as from the point *a* to point *y*, the conidium may be said to be subcylindrical.

Mere casual observation of many strains of Helminthosporium showed that the point of maximum diameter was usually near the basal region of the conidium, occasionally near the middle region, while in extremely rare cases it was near the apical quarter, the ratio of these cases being for H. No. 1 about 30:14:4. A more accurate determination of what may be termed the longitudinal eccentricity of the conidium—that is the range of variation in the position of the line of greatest diameter (a—a', Diagr. I—IV) may be made by measuring (along the longitudinal axis) the distance from the base of the conidium to the intersection of the line a—a' with the longitudinal axis. This distance divided by the total length of the conidium may be known as its *coefficient of longitudinal eccentricity*. This coefficient for H. No. 1, based on 65 conidia taken at random, was found by the above method to be $.43 \pm 0$. In other terms the point of maximum diameter was distant from the base of the conidium 43% of the total length of the conidium. Bakke (6) says that the conidia of *H. teres* are widest at the middle. The coefficient of longitudinal eccentricity based on 11 conidia of H. No. 1 which were of typical subcylindrical appearance (approaching that shown in Diagr. IV) was .45 as contrasted with a coefficient of .43 for 11 conidia of elliptical appearance (Diagr. I). Coefficients of longitudinal eccentricity for H. Nos. 5, 20, and 4 of subcylindrical shape, were respectively .35, .39, and .37, showing that in these forms the point of maximum diameter is slightly nearer the base than it is in H. No. 1. None of the conidia of H. No. 1 was truly cylindrical, that is, the sides were not parallel for any appreciable distance. Many were subcylindrical, the form approaching that shown in Diagram IV. Of 65 conidia taken at random 81%+ of the conidia were elliptical; 17%+ subcylindrical; and 1% otherwise.

To secure a coefficient which would indicate with some degree of accuracy the curvature of the conidial wall (as from point a to point y, Diagr. I—IV) determinations were made of the ratio $\frac{xy}{gh}$ (Diagr. I—IV). The line cd was tangential to the surface of the conidium at the point of maximum diameter, and was parallel to the longitudinal axis of the conidium, the line ef being 3.4 μ from the line cd and parallel to it. Then the points x and y are where the surface-line of the conidium cuts the line ef. It is obvious that as the line xy increases in proportion to the length of the conidium, gh, the conidium more nearly approaches the form of a cylinder; and as the line xy becomes proportionately shorter the conidium becomes less like a cylinder. The ratio $\frac{xy}{gh}$ may therefore be termed the coefficient

of cylindricity. For these determinations only conidia of approximately modal length were used, and to obviate unconscious selection, measurements were made of only the left side of the conidium, the basal end being toward the observer. Determination from 11 conidia of H. No. 1 of subcylindrical shape gave a coefficient of .74, while that from 53 elliptical conidia was .67.

The above findings for H. No. 1 are as follows:

Coefficient of longitudinal eccentricity	
All conidia	.43
Elliptical conidia	.42
Subcylindrical conidia	.45
Coefficient of cylindricity	
All conidia	.70
Elliptical conidia	.67
Subcylindrical conidia	.74

Determinations of the coefficient of cylindricity made from drawings of Dr. Ravn (91) gave for *H. gramineum* and *H. avenae* respectively .86 and .95, showing a much higher coefficient than is given by any of the forms in my collection.

A convenient method of measuring conidia for coefficients is given on page 179 of the appendix.

Conidial length.—From five separate plates, *a*, *b*, *c*, *d*, and *e*, inoculated with H. No. 1 under standard conditions, Graphs 36-40 (Fig. K) of conidial length were made. Two additional graphs were made from plate *e*, one of which is designated as *e'*. The data pertaining to these graphs are given with the others (Fig. K).

The differences between the means of conidial length on plates *a* to *e* and *e'* are as follows:

Plates	*Differences between means*	*Plates*	*Differences between means*
a−*b*	+0.70 ± .21	*b*−*e'*	−0.19 ± .16
a−*c*	+0.62 ± .24	*c*−*d*	+0.16 ± .23
a−*d*	−0.78 ± .23	*c*−*e*	+1.40 ± .24
a−*e*	+2.03 ± .24	*c*−*e'*	+0.11 ± .17
a−*e'*	+0.50 ± .16	*d*−*e*	+1.24 ± .25
b−*c*	−0.07 ± .22	*d*−*e'*	−0.28 ± .18
b−*d*	+0.08 ± .23	*e*−*e'*	−1.52 ± .19
b−*e*	+1.32 ± .24		

Since the various plantings on these plates were all from the same inoculum, made at the same time, and under as nearly identical conditions as possible, and so kept, the rather large difference in means seen, particu-

larly in *a*—*e*, *b*—*e*, *c*—*e*, *d*—*e*, and *e*—*e'* is significant. If plate *e* be left out of consideration, the others agree reasonably well, with differences greater than the probable error in six out of ten cases, the difference being but slightly above the probable error in two cases, about twice the probable error in two cases; and about thrice that, in two cases, the largest excess, in plates *a*—*b*, being 0.70 ± .21.

Plate *e* deviates widely, with a difference in case of *a*—*e* of 2.03 ± 24, the difference being more than eight times the probable error. The great difference in plate *e* must indicate variability of the fungus on this plate (cf. with page 152), modification due to influence of some unknown factor of environment, or error in sampling. But since such a variation did occur in a series of plates made with the greatest care and with the same organism, it is clear that the occurrence of such a difference can not properly be interpreted as meaning specific difference. Data from the combined records of *a*, *b*, *c*, *d*, and *e'* (omitting *e* as questionable) give the most reliable data I have on length of conidia of H. No. 1 under standard conditions (cf. with Graph 42, Fig. K).

To determine how wide a variability occurs in specimens collected in the open, on the natural host, *H. ravenelii* (Pl. XX) a well-marked, easily recognized species of wide geographic distribution, growing on Sporobolus, was studied in conidial-length graphs made from specimens listed in connection with the graphs (Fig. L). The tabulated results of this study of *H. ravenelii* follow:

*Nos.**	*Differences between means*	*Nos.**	*Differences between means*
43—46	0.48 ± .26	46—50	0.50 ± .28
43—47	0.59 ± .24	46—51	0.70 ± .28
43—49	0.62 ± .28	46—52	1.46 ± .28
43—48	0.62 ± .29	46—53	2.43 ± .27
43—50	0.98 ± .27	47—51	0.59 ± .26
43—51	1.18 ± .27	47—52	1.35 ± .26
43—52	1.94 ± .27	47—53	2.33 ± .25
43—53	2.92 ± .26	48—51	0.56 ± .30
44—48	0.45 ± .31	48—52	1.32 ± .30
44—49	0.46 ± .31	48—53	2.30 ± .29
44—50	0.81 ± .30	49—51	0.55 ± .29
44—51	1.01 ± .30	49—52	1.31 ± .29
44—52	1.77 ± .30	49—53	2.29 ± .29
44—53	2.75 ± .29	50—52	0.96 ± .29
45—50	0.80 ± .24	51—52	0.76 ± .29
45—51	1.00 ± .25	51—53	1.73 ± .28
45—52	1.76 ± .25	52—53	0.97 ± .28
45—53	2.74 ± .24		

*For significance of numbers, see Figure L.

A difference of 0.97 ± .28 between the means of two samples from the same specimen (Nos. 52 and 53)—a difference more than three times the probable error—shows clearly the difficulties of sampling, and that such differences between samples of the same species grown under the same conditions may be expected. The differences in several instances, notably between Nos. 44 and 49, 44 and 48, and 46 and 51, are no greater than those between two samples of the same specimen and may well be due to sampling, and to this extent show the fungus, over a wide geographic range, to be remarkably uniform. In several other instances, however, there is a wide difference of means, above the probable error—notably in all cases involving sample No. 53. These differences are often four, five, or six times the probable error, and occasionally run as high as eleven or twelve times the probable error even with this remarkably uniform fungus. While these differences may in part be attributed to sampling they probably represent also morphological changes due to environmental differences, and differences of nutrition or humidity, but do not necessarily indicate racial difference in the fungus.

To determine whether various cereals, autoclaved, influence conidial length differently, plates of H. No. 1 were prepared under standard conditions except that in the same Petri dishes were placed shoots of wheat, rye, barley and corn. The resulting graphs of conidial length are given in Fig. M. The differences in means are as follows:

On rye and wheat, 0.40 ± .29
On rye and corn, 0.45 ± .20
On wheat and corn, 0.02 ± .22

The mean length on rye, corn, and barley is in close agreement with that on wheat, and, apparently, under these conditions the species of shoots counts for little in its influence on conidial length.

Conidial-length graphs (Fig. N) made from H. No. 1 grown on fresh wheat-stems, on young wheat shoots, on wheat leaves, and on young wheat plants, all autoclaved in test-tubes with a few centimeters of water, show a considerable increase over those under standard conditions (Graph 42, Fig. K); also, in Graphs 58, 60, and 61 (Fig. N), they show a much larger standard deviation and coefficient of variability, probably due to the variable humidity under these conditions. The small number of conidia measured, and the lack of control over humidity may be presumed to account for such variation as is seen.

Live wheat inoculated in rag doll showed at the 6th day 100% infection. These infected seedlings were placed in a Petri dish on moist filter-

paper and the conidia allowed to develop to maturity. Conidial length here (Graph 64, Fig. O) was somewhat less than under standard conditions (see Graph 42, Fig. K), and the coefficient of variability was a little high.

Conidial breadth.—H. No. 1 was quite constant in conidial breadth as follows:

M	σ	CV
$6.03 \pm .04$	$0.55 \pm .34$	$9.13 \pm .57$

The ratio of conidial length to conidial breadth is an important one as determinative of shape. This ratio for H. No. 1 is as follows:

$$\frac{\text{mean length}}{\text{mean breadth}} = \frac{22.62 \pm .05}{6.03 \pm .04} = 3.74 \pm .03$$

In a description of H. No. 1, written in May, 1919, for my own use, and prepared with considerably more care than is ordinarily used in specific descriptions of fungi, I noted the conidia as 3—8 septate and as 52.6—67.2 $\times$19.2—24 μ long on wheat; and as 48—84$\times$18—21.6 μ on corn-meal agar, whereas my more extended study now shows the mode on wheat as 78.2 μ, the mean as 76.8 μ, and the range from 34 to 98.6 μ; the breadth as ranging from 17 to 23.8 μ, with the mean as 20.4 μ; the septa with a mode of 8, a mean of 7.9, and ranging from 4 to 10. I may here note also that Bakke (6) in his description of *H. teres* gives the conidial dimensions as 150 [or 105*]—130$\times$15—20 μ, and the septa as 7—14. Thus he seems to have found conidia considerably longer than I did, as also narrower ones. It should be said that the data obtained by this study of graphs of H. No. 1, though involving several thousand measurements, fail to record the longest conidium observed, and the one with the most septa, because these were both seen during observations which rendered their inclusion impossible; which is to say that to include them would have been to consciously *select* these unique conidia for inclusion. Anent the shortcoming of my own brief description cited above may be quoted the Saccardian description of *H. ravenelii:* "Spongiosum; hyphis flaccidis flexuosis nodosis ramosis, inarticulatis; conidiis cymbiformibus, 3-4 septatis, fuscis, 50 μ longis, endo-chromotibus isthmo connexis." Though the mode is approximately at 50-54 μ the conidia really range from 13 to 71μ (see Fig. L). Very similar errors, due to brevity of description, exist regarding many or all known species.

*See Pammel, King, and Bakke (90, p. 180).

Etiology of Foot-rot

evidences of etiological relation of h. no. 1

Constant Presence of the Pathogene

In all cases of American foot-rot of wheat that have come under my observation the rotten basal portion of the shoot bore and was to a large extent occupied by a mycelium, which grew luxuriantly within the wheat tissue though very sparingly upon its surface, coursing lengthwise within the diseased cells. This mycelium was hyaline, septate, vacuolate, irregular in thickness, and, in short, agreed in all characters with those of H. No. 1 when growing in rotting wheat-tissue (page 105).

Absence of other Constant Parasites

No other organism which might be considered as a possible parasite was present in any large number of cases in or on the wheat tissue. Amebae and nematodes were present in great numbers in the soil, but appeared to bear no relation to the rot of the wheat. Various fungi, as Fusarium (two species), Rhizoctonia, Epicoccum, Alternaria, were occasionally found on the roots or stems, but each only rarely, in a fraction of 1% of the cases, and with no evidence of etiological relation to the rot of the stem.

Identity of Pathogene proved by Culture

Very numerous isolations were made by taking bits of tissue (1) from diseased sheaths, (2) from diseased stem-lesions, and (3) by stripping away the sheath, disinfecting the remaining surface with mercuric chloride and taking out diseased bits, with precautions against contamination. All such diseased bits were laid on the surface of corn-meal agar plates. Hundreds of these were made, with the result that in practically every instance the diseased bit gave rise to Helminthosporium conidia in general aspect like those of H. No. 1. Other organisms, as mentioned, occasionally occurred on these plates, but in only a small per cent. of instances. It seems entirely conclusive that the mycelium constantly found in the rotting basal portion of the diseased wheat-stems is that of a Helminthosporium.

Evidence of Infectiousness

Several bags of soil that bore diseased wheat in 1919, near Granite City, Illinois, were brought into our greenhouse in July, 1919. In this soil was planted "Sultzer Pride" wheat, and the planting kept liberally watered. At the end of some weeks the plants were removed, and on examination all

showed browning and incipient rot of the basal portion of the stem. Microscopic examination and agar platings from these stems gave results identical with those stated above. One plant that was so badly rotted in the pot as to fall over was found bearing Helminthosporium conidia on its surface.

Conidia produced in Moist-chamber Culture

While stems with diseased lesions, either from the field or greenhouse, when placed in an ordinary moist-chamber rarely gave Helminthosporium conidia (or, if they did, only in small numbers), if the diseased stems were placed on wet filter-paper in moist chamber and rather closely covered with wet filter-paper Helminthosporium conidia invariably developed in quantity on the lesions, the fungus eventually spreading throughout the available wheat-tissue and producing conidia over the whole surface (cf. with page 95).

Evidence from Inoculation

Severed, live wheat-shoots, grown under aseptic conditions, were placed as under standard conditions (Appendix, page 180), except that the shoots were not autoclaved but put, living, upon the inoculated agar. All such shoots rotted rapidly and completely, the shoot being eventually covered by Helminthosporium conidia. Since direct examination showed no contamination, it is evident that H. No. 1 can cause rot of the wheat tissue.

To determine the relative rotting power of this organism and other Helminthosporiums under these conditions, fresh aseptic shoots of corn, wheat, oats, barley, and rye were laid on washed agar with the growing tip toward the circumference of the dish, and the cut end in contact with the outer edge of the spreading mycelium of a colony about 5 cm. in diameter. These were examined after 2 days and again after 5 days, and the rate of browning was carefully calculated. In this way seventeen strains of Helminthosporium were tested as to their ability to produce rot in live, severed cereal-shoots. H. No. 1, the foot-rot organism, showed high rotting ability, completely rotting a wheat shoot 11 mm. long in 5 days, while H. No. 2 (*H. ravenelii*) produced no rot on any cereal. H. No. 1 rotted corn also, but much less rapidly than it did wheat, and its rate on oats, barley, and rye was still less. Several other numbers showed strong rotting power on wheat shoots, notably H. No. 10 (labeled *H. teres*), isolated by Dr. Stakman from barley, H. No. 9 isolated by him from wheat, and H. No. 13 (labeled *H. sativum*), isolated by Dr. Durrell from barley.

The results from this preliminary work indicate also a very wide difference in the susceptibility of these cereals to rot by the various strains of Helminthosporium. Oats, on the whole, are less injured by them than any of the other four cereals tested. Corn and wheat were most often first in susceptibility to certain of the strains, and were also highly susceptible to more strains than were barley and rye.

Seedlings in Petri dishes inoculated.—Aseptic wheat-seedlings were placed on moist filter-paper in sterile Petri-dishes and were inoculated in their basal region in three ways: by placing upon them (1) wheat tissue rotted by H. No. 1 (pure culture), (2) conidia of this organism, and (3) agar bearing an abundance of growing mycelium. No difference was observed in the effectiveness of the three modes of inoculation. Each gave a 100% infection, always visible with a hand lens in 2 days (Fig. 16) as a small spot, which could usually be seen at the same time without a glass. A longer time than two days was necessary to demonstrate that this spot would develop into a general rot, but so it did in all cases when the environment was favorable.

Seedlings in rag doll inoculated.—Wheat seedlings with shoots 2-3 cm. long were placed in a special form of rag doll (Pl. XXXIII) and inoculated with H. No. 1 by placing an oese of conidia-suspension on the base of each shoot without wounding. Infection was apparent to the naked eye in every case in two days, and the results in six days are shown in Pl. XXXIV. Rotting occurred in 6-12 days under favorable conditions. At 6 days the roots were often more or less blackened for long distances and the cortex filled with mycelium. Views of cross-sections showed a heavy infection of the second leaf, and the sheath completely occupied. With excessive moisture, seedlings were killed by the Helminthosporium in 6 days; but if in comparative dryness, only small lesions resulted. Seedlings similarly placed in rag-doll but atomized with conidia-suspension also gave 100% infection, and the infection was much more widely distributed.

Inoculation by diseased tissue or by fungus-bearing agar was in no way superior to inoculation with conidia.

Control, or check, rag-dolls, made in the same manner but without inoculum, at 2 and 6 days showed no lesions even under microscopic examination. In a very small number of cases there was infection by Helminthosporium in the checks, and in a few instances overgrowth by a Helminthosporium similar to H. No. 29, with geniculate conidia.

Roots of wheat inoculated.—Conidia of H. No. 1 were placed on the root-hairs of wheat-seedlings in rag doll. At the end of 4 days all roots so in-

oculated were yellowish or pale straw-color, as contrasted with the white, uninoculated roots, and they had scant root-hairs. Under the microscope

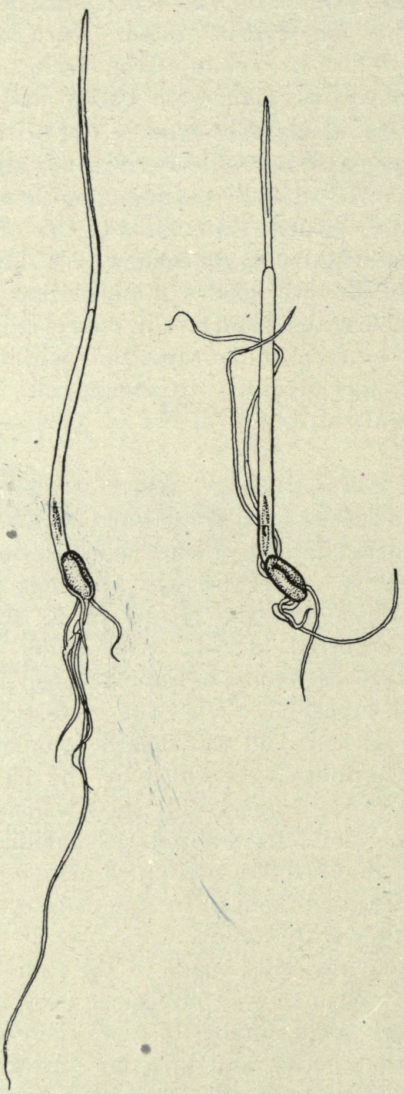


FIG. 16.—Lesions on unwounded wheat-seedlings two days after inoculation with conidia of H. No. 1. The shaded portion of the shoot was yellow to brown.

the cortical tissue was seen to be crowded with Helminthosporium mycelium coursing mainly in the longitudinal direction of the root. The mycelial threads within the root cortex were remarkably thick—13μ. Wheat seedlings 2 cm. long, atomized with conidia suspension of H. No. 1, in 6 days were covered with infection spots over their whole surface.

Inoculations in soil.—Vials 12×70 mm., prepared as described on page 180, were used as containers. Wheat seeds were germinated aseptically, and when the shoot was about 2 cm. long they were inoculated and transferred to the soil in a vial. The results differed in no essential way from those described for the rag-doll inoculations, though the plant could be kept longer under observation since it was not solely dependent on the seed for food. Aseptic wheat-grains were also planted in these vials with the inoculum placed in three different positions: (*a*) on the seed; (*b*) 1.5 cm. above the seed; (*c*) 1.5 cm. below the seed. When on the seed, lesions occurred low; when above the seed, they were higher; when below the seed, no lesions were on the stem in early days but the roots were heavily infected.

Duplication, in pots and in benches, of all the above experiments made in vials gave identical results.

Recovery of Organism

After all the types of inoculation mentioned above, the organism used in the inoculation was clearly evident in the tissues and producing conidia upon them, and by dilution-plating it was recovered from them. During such recovery there was sometimes evidence of bacterial or other contamination, but in most cases of each type of inoculation no contamination occurred, and the pathogenic changes noted were clearly attributable to the fungus used in the inoculation.

INFECTION PHENOMENA ON WHEAT

Conidia of H. No. 1 and of H. No. 14 when placed on wheat in rag doll germinated from one or both ends as described elsewhere. The germ-tube grew rapidly, branching freely, and oriented itself lengthwise of the shoot more frequently than crosswise or obliquely, often following lengthwise the boundary between two wheat-cells. At certain places where this mycelium touched the wheat-surface it swelled slightly, producing a round or oblong *appressorium*. These appresoria sometimes, probably most often, arose by the simple swelling of a cell of the main thread (Fig. 17), though frequently also from short lateral branches (Fig. 17, *d*) or where the terminal

cell of a thread abutted against the wheat tissue (Fig. 17, *g*). So far as observed they differed from the usual mycelila cells only in shape. The appressoria are very numerous (Fig. 17, *b*). They are usually produced only after the mycelium has grown to considerable length; not, as is the case with some fungi, immediately on emergence from the conidium. In

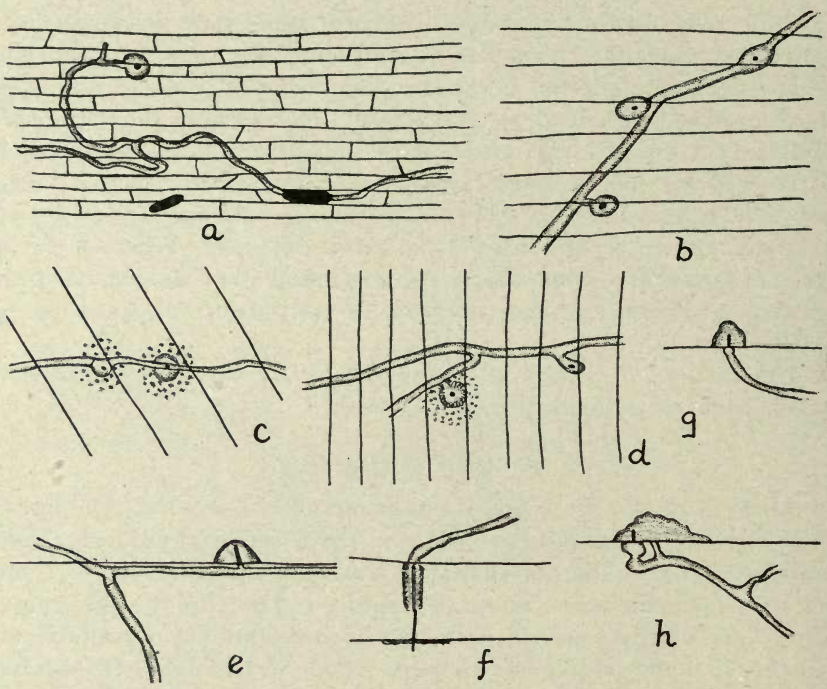


FIG. 17: *a*, H. No. 1 on wheat, 24 hours after inoculation, showing mycelium arising from a conidium, an appressorium, and penetrating mycelium; *b*, *c*, *d*, H. No. 14, showing appressoria, penetrating points, and "callus"; *e*, *f*, *g*, *h*, H. No. 1: *e*, mycelium within cell, and with a penetrating mycelium reaching into an adjacent cell, a "callus" there resulting; *f*, mycelium ending squarely against a cell-surface, penetrating it and then being covered by "callus", and eventually penetrating this and the next cell-wall, the latter being thickened; *g* and *h*, similar to *e*.

most cases the penetrating mycelium, viewed from above, appears as a minute bright point, or as if a minute hole had been pierced in the wheat cell-wall, much as is seen in the hyphopodia of Meliola (82) or on the appressoria of Gloeosporium (64) where penetration organs arise. Viewed laterally, the bright point of the appressorium is seen to mark the emergence of a haustorium-like strand which I shall continue to call the penetrating mycelium. This structure is much thinner than the usual mycelium (see Fig. 18) and of different staining reactions. It penetrates the wheat

cell-wall, and is sometimes simple, sometimes branched. At the place where the penetrating mycelium pierces a wall and enters a healthy wheat-cell there is developed, on the inside of the wheat-cell and surrounding and covering the penetrating mycelium, a callus-like structure (Fig. 17, *e-g*) which for brevity I shall term the "callus". As the penetrating mycelium continues to grow, the "callus" grows *pari passu*. Where many penetrating mycelia develop near each other this "callus" may become very large

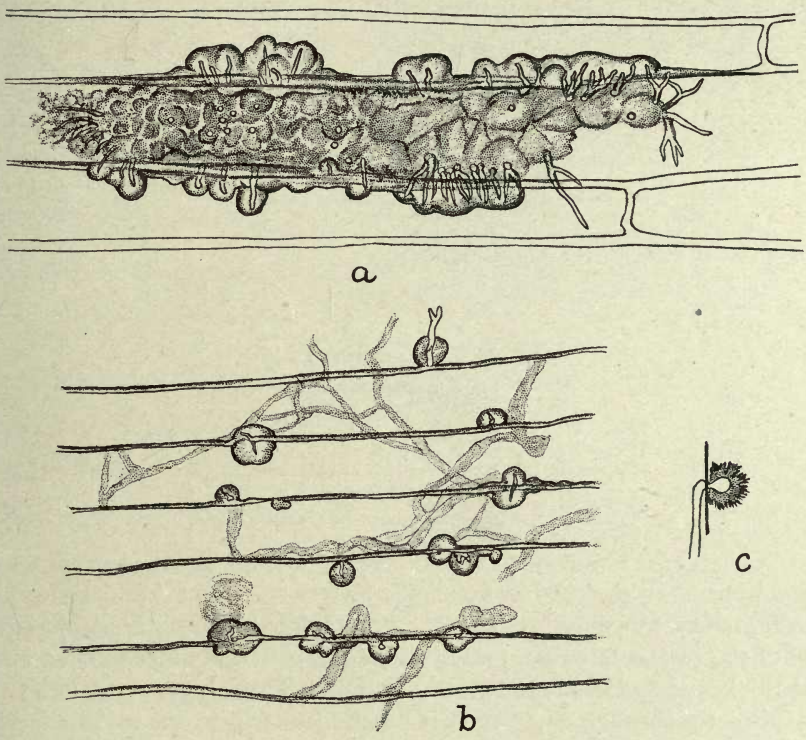


FIG. 18.—H. No. 1: *a*, large "callus"-formation, with many penetrating mycelia piercing the cell walls; *b*, mycelium spreading over the wheat surface, and at many contact points producing appressoria and penetrating mycelia; *c*, penetrating mycelium of unusual form, and the "callus" rough.

(Fig. 18, *a*) and complicated. The "callus" formation seems to be of the nature of a precipitation, probably resulting from toxic action, and a badly intoxicated cell can, in its protoplasmic disorganization, make numerous such deposits at points other than those of mycelial entrance. Thus in some instances the whole inner surface of a cell's walls may be thickly

studded with small dewy drops, apparently of precisely the same character as the "callus." (See Fig. 23, page 135.)

The host's cell-wall at and near the point of penetration, is markedly altered chemically, as is shown by various stain-reactions. Thus, adjacent to the point of infection several different regions giving different chemical reactions may be distinguished, as is indicated in Fig. 19. Region 3 gives the usual chlor-zinc-iodide reaction and stains like normal cellulose. None of the other regions do this. Region 4 stains darker with the usual

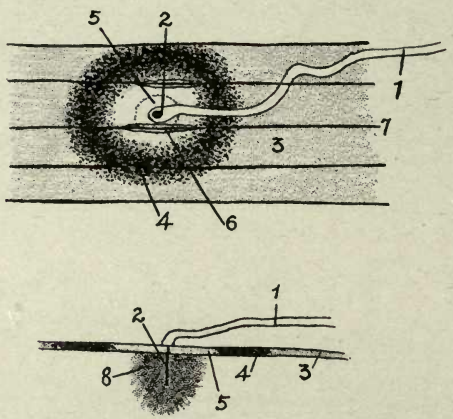


FIG. 19.—H. No. 1: regions of a young diseased spot: 1, mycelium; 2, penetrating mycelium; 3, normal wheat cell-wall; 4, region of darker staining; 5, region of lighter staining; 6, diseased inner lamella; 7, middle lamella; 8, "callus."

stains, but not so dark as normal cell-wall. The "callus" and penetrating mycelium stain faintly or not at all. The middle lamella stands out clearly in all of the diseased region, and on each side of it the inner lamella is seen to be thickened and of altered stain-reaction. Though penetration is sometimes directly through the wall it is much oftener into the middle lamella, and the mycelium shows a strong tendency to follow along the line of division between two cells, thus giving a gridiron effect to the mesh. This is possibly due to chemotropic attraction by the middle lamella or, possibly, because this is the weakest place in the cuticle. No case of stomatal entrance was observed; indeed, on the sheaths of "Golden Chaff" wheat stomata are seldom present.

Once within the host cell the mycelium grows rapidly, soon nearly or completely filling it (Fig. 20), and often forming a mass so dense that it resembles a pseudoparenchyma. Both longitudinal and transverse sections

show clearly that the mycelium is within, not between, the host cells. Penetration into adjoining live cells is attended by the same phenomena of penetrating mycelium, "callus" formation, and wall-changes, though appressoria were not observed in such cases, possibly on account of the difficulty of observation. Penetration into dead cells is not attended by these phenomena.

The chronological history of a lesion from a simple infection begins with the attack on one cell, which is soon overcome and occupied, and at 24, or, better, 48 hours after inoculation, observation with a 16 mm. objective shows regions with one to several cells diseased and browned,

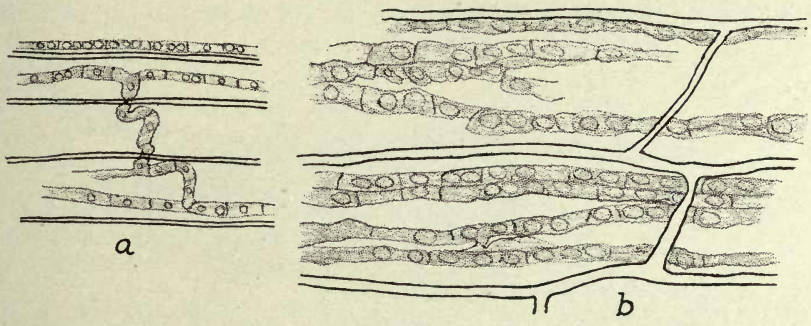


FIG. 20.—H. No. 1 on wheat: *a*, mycelium in cells and penetrating the side walls; *b*, mycelium running lengthwise within the wheat cells.

and the protoplasts undergoing disorganization and becoming browned. Owing to the length of the wheat-cells, the diseased regions are much longer than broad, and in many instances two diseased cells or two rows of them are seen with a quite healthy cell between them (Fig. 21). Under action of Javelle water the healthy cells plasmolize beautifully, while the sick cells show no plasmolysis. Treated with acid fuchsin in glycerine, normal cells show no stain, while in diseased cells the entire protoplast becomes pink and the inner lamella, which is swollen, also stains pink. This softening and swelling of the lamellae was extensively studied by de Bary (8), Ward (123) and Büsgen (30). De Bary, who, in 1886, was first to separate a cytolytic enzyme from fungi (*Sclerotinia libertiana*), states that as the inner lamellae undergo partial dissolution they continue for a time to give the cellulose reaction, but eventually swell, disorganize, and lose this property (8, page 420). He also describes the fungus as growing in the middle lamella. Ward (123) describes the cellulose as swelling and softening under action of the enzyme produced by Botrytis. Here, too, the

mycelium grows in the middle lamella. Jones (71), working with *Bacillus carotovorus*, reports that the enzyme produced, attacks more strongly the middle lamella, but he noted also a softening and swelling of the inner lamella, but found that the cellulose stains (e. g., chlor-zinc-iodide) "give clear blue reactions with these fully softened walls." Van Hall (63), working with *Bacillus omnivorus* on Iris, reports a similar condition. The inner lamellae, swollen by Helminthosporium, no longer react as cellulose under this test. Blackman and Welsford (18), who describe in detail the entrance of *Botrytis cinerea* into bean leaves, state that neither before nor after penetration did the staining reactions of the cuticle give any evidence of its being softened or swollen or in any way altered chemically (though the subcuticular walls usually, if not always, swell), and no swelling

FIG. 21.—H. No. 1 on wheat shoots, second day after inoculation. Shaded portion was colored brown.

of the subcuticular cellulose was observed before the passage of the invading hypha through the cuticle. Pathogenic changes in the inner lamella precede those in the protoplast, that is, no toxin acts upon the protoplast prior to the swelling of the lamellae. The subcuticular layer swells. Penetration of the cuticle is by pressure. Gardner (58) mentions no changes occurring normally in staining reaction of host cellulose in leaves attacked by Colletotrichum, though in cases of delayed penetration he notes that the cell-wall under the appressorium retained safranin better than did normal cell-walls. In fruit penetration, however, he found that, characteristically, the inner lamella was so altered as to retain safranin. The action appears to be different in both quality and quantity from that described by Newcomb (86), who, studying enzymes in seeds, states that "with all the ferments the walls at first become hyaline, appear

gradually more transparent and finally 'melt away.' " In Colletotrichum Gardner (58) found the fungus similarly seeking the "depressions bounding the epidermal cells." This place of entrance is characteristic of many fungi—see Büsgen (30), Behrens (15), Ward (123), Noack (87), Miyoshi (83), Nordhausen (88), Schellenberg (99), and Aderhold (1). The last three named, believe this to be due to chemotaxic influences. Noack in describing the entrance of *H. gramineum* into the host mentions the appressoria. Similar structures have also been described in the anthracnose fungi by Hasselbring (64) and by Gardner (58), the pore in these structures being such as I find in Helminthosporium, though the appressorium in the anthracnose fungi is a mere swelling and is hyaline. Similar extreme narrowness of the mycelium at the actual point of penetration of host-walls is shown

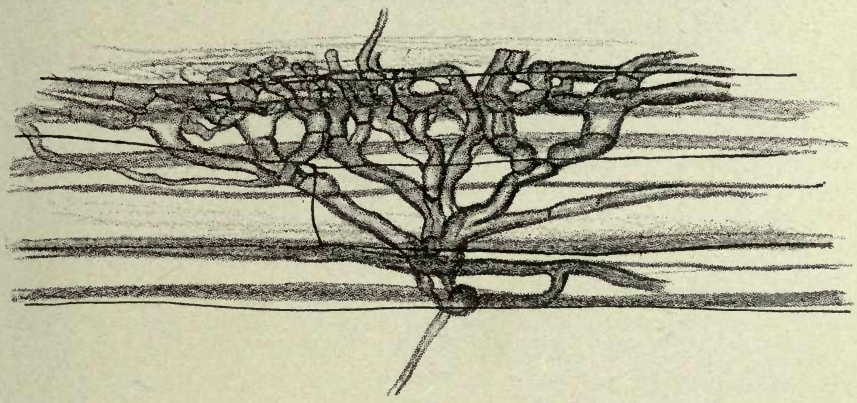


FIG. 22.—H. No. 14 on wheat, showing fan-like mode of branching, see p. 105.

also by Ward (123, fig. 57), Gardner (58, page 27), Hasselbring (64), Büsgen (30), and Noack (87). Bakke (6) says of *H. teres* that the mycelium "penetrated the epidermis directly and made its way through the intercellular spaces," but he gives no further details.

Conditions very closely resembling the "callus" formation are figured by Dastur (41, figs. 8, 9), depicting the entrance of smut into sugarcane. This appears to have occurred only occasionally, and Dastur regards the "callus" ("plug") as probably a means of preventing infection. Conditions somewhat resembling that of the "callus" formation are described and figured by Wolff (128, figs. 2, 3) and by Brefeld (25, fig. 2) in the penetration of smuts into cereal tissue. Wolff (128, p. 20) describing this says: "Es tritt hierbei der eigenthümliche Umstand ein,

dass der Faden, sobald seine Spitze in das Innere der Zelle tritt, nicht frei in dieses hineinwächst, sondern von den inneren Schichten der Zellwand, welche sich gleichsam aus stülpen, wie in eine Scheide von bald grösserer bald geringerer, oft sehr beträchtlicher Stärke eingeschlossen wird und in dieser bis zur nächsten Zellwand weiter wächst." Brefeld describes very similar conditions, including much thickening which is of yellow color, but instead of interpreting it as an enclosing sheath he regards it as wholly due to thickening of the walls of the mycelium itself. He moreover states that this phenomenon is indicative of conditions in the host, as too great age, that are unsuitable to infection, and that it is not in evidence when the host is in fully susceptible condition. Whichever may be the true interpretation in the case of cereal smuts, I am convinced that in case of Helminthosporium the "callus" is produced by

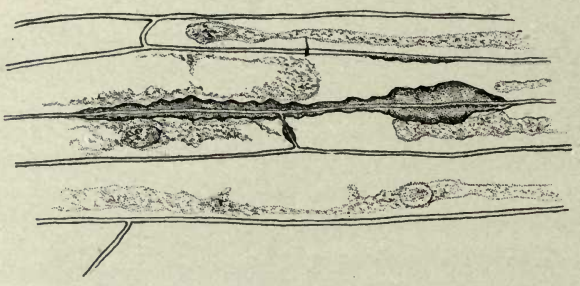


FIG. 23.—Infection by H. No. 1, 24 hours after inoculation, showing thickening of the wheat cell-walls by deposition on their inner surfaces. (Text citation at top of p. 131.)

the wheat-cell, and is not part of the mycelium. Ravn (91), describing the reactions to the intercellular mycelium of Helminthosporium in cereals, states that a thickening appears upon the cell-wall of the host, resembling a drop segregated from the cell, and that several such thickenings may be seen upon one cell, sometimes filling the intercellular spaces completely. They seem to differ from those that I describe (Fig. 17), however, in position, since they are without, not within, the cell, and in composition, as those noted by Ravn take aniline stains readily.

Ravn (91, fig. 23) describes an appressorium very much like that which I find and states that the mycelium from it enters the epidermal cell, where it so increases that it may fill the cell; then makes its way to the intercellular spaces and grows there exclusively, never again entering any of the cells even by means of haustoria. It therefore appears from his statements and figures that the Helminthosporiums with which he

worked, differed in a very fundamental way, as pathogenes, from those which I am studying, his forms being intracellular (except as regards the first cell invaded), and not at once killing the adjacent cells. That is, the condition pictured is much like that presented by Albugo, Peronospora, Puccinia, etc., except for the absence of haustoria. The forms with which I deal, on the other hand, though they enter through the middle lamellae, immediately become intracellular and at once kill the protoplast of the invaded cell, and proceed similarly with other cells. These differing conditions, if substantiated by further study, probably indicate fundamental differences in the fungi in regard to their production of toxins or enzymes, and certainly indicate an entirely different type of pathogenicity. In these early stages the disease is properly a spot and not a rot. Whether it will develop into a true, general rot depends upon conditions. Phenomena like those described under the present heading, though differing in detail, were noted with H. Nos. 6, 8, 9, 14, 21, 36, 39, 40, and 41.

Action of various strains of Helminthosporium on wheat shoots.—Tests in rag doll, at medium moisture, with H. No. 1 and H. No. 3 gave at 2 days 100% infection for both; at 6 days there was no appreciable difference between the two; while at 10 days all shoots were rotten under H. No. 1 and some, but not so many, under H. No. 3. The test was repeated with 14 strains of Helminthosporium. All strains at 2 days showed 100% infection; the controls, no infection. The infection phenomena with all of these strains were all of the character described on pages 128, 129, showing penetrating mycelium, "callus," etc. At 6 days H. Nos. 1, 4, 5, 8, 13-16, 20, and 21 had all produced some rot. The roots also were distinctly yellowed by H. Nos. 15 and 16, while H. No. 20 showed less rotting than the other numbers mentioned above. H. Nos. 29 and 39 produced no rotting, and the lesions were visible only through a lens, but thus viewed, showed 100% infection, as indicated by the usual infection phenomena. H. Nos. 3, 6, 9, 17, and 18 remained local, as at 2 days. H. No. 29, a Helminthosporium with geniculate conidia, germinated abundantly from both ends of the conidium, and on wheat produced many penetrating mycelia and an abundant mycelium within the host, though the mycelial invasion reached only a few cells, and while extending for a considerable distance lengthwise, made but little progress laterally. The appressoria were usually pyriform, as was also the penetrating mycelium, differing thus from H. No. 1 (Fig. 17). Similar tests were made with three saltants, M6, M8, and M38. Notes at 2 days showed 100% infection, and at 6 days much rot by M6, and considerable rot by the other two.

Though infection can be determined with certainty, I have as yet no means of accurately measuring rotting power, or of determining whether differences noted in rotting are due to environment, host, or fungus. It seams clear, however, that H. No. 29 is capable of causing only local spotting; and that the other numbers, perhaps even the saltants, vary somewhat among themselves in rotting capacity, most of them causing rotting to some extent under favoring conditions.

The fact that so many and diverse races of Helminthosporium are able to cause rot of wheat, led me to test the ability of Alternaria to parasitize wheat seedlings. An Alternaria, found commonly on wheat seed, was isolated and inoculation made in rag doll on wheat seedlings. At 24 hours many wheat cells showed diseased spots, being in every way like those described on pages 128, 129, including the swollen middle and inner lamellae, browning of the cell-contents, and formation of the "callus" and penetrating mycelium. The Alternaria mycelium crossing several middle lamellae, usually produced an appressorium and penetration at each middle lamella. The Alternaria mycelium was also seen to enter the wheat-cells, killing a few of them, but in no instance was this fungus observed to cause rotting or to produce a spot large enough to be visible to the naked eye. It was seen, however, to penetrate the cells of the root cortex quite extensively, causing a slight browning. Sterigmatocystis, Penicillium, and several other fungi supposed to be mere saprophytes, were treated in similar manner, but produced none of the phenomena of infection.

SUSCEPTIBILITY OF VARIOUS HOSTS TO INFECTION

Tests in rag doll with H. No. 1

Corn.—Three seedlings showed no infection at 2 days, though conidia were present and had germinated. At 6 days all three plants were infected, the infection being confined to one or two cells, though the mycelium was clearly evident in these. Pammel, King, and Bakke (90) report negative results regarding infection trials of *H. sativum* on corn, but their tests were limited to leaves.

Barley.—At two days one plant was slightly infected, showing several lesions. In these the mycelium was abundant within the cells. Eight were not infected. At 6 days the infection showed no further progress.

Rye.—At two days three plants were infected; six not infected. At 6 days the infection showed no progress. The mycelium was observed within the cells and infection phenomena were as on wheat.

Sorghum (*Holcus sp.*).—There was 100% infection of both roots and stems, with pronounced rot. The same phenomena were observed as on wheat, including the appressoria, penetrating mycelium, and "callus." The sap of the infected cells was strongly tinged with red, and the "callus" and appressorium were deep red. Adjacent colorless walls soon became swollen and reddened. The red coloring-matter is absorbed by the nucleus, which becomes as brilliantly colored as by an aniline dye. At six days the shoots and roots were heavily infected, the diseased regions assuming a deep red, almost black, color, and conidia formed abundantly over the lesions. When such specimens were placed in alcohol, the red color diffused to the alcohol, coloring it strongly. This red coloration by the host is a response common on invasion of either bacteria or fungi on the sorghums and sugar-cane, and on corn in the case of some diseases.

Sudan grass (*Holcus sorghum sudanensis*).—At ten days 1 seedling gave positive and 5 gave negative results; at six days, 5 positive and 4 negative. Infection was slight on a few cells, but the mycelium was evident within the cells, and infection phenomena as on wheat were observed.

Common millet (*Chaetochloa italica*).—At two days 10 gave positive results. At six days the rot was progressing into the roots faster than into the stem, though black spots 3—4 cm. long were apparent. Infection phenomena were observed as on wheat, and much mycelium was seen within the tissue.

German millet (*Chaetochloa italica germanica*).—The results were practically the same as with common millet.

Amber cane (*Holcus sorghum*).—Results were much as on sorghum.

Red top (*Agrostis palustris*).—No phenomena of infection were observed.

Beans.—No rot was produced, no "callus", nor any other of the usual signs of infection; nor was it certainly determined that the mycelium entered the host-cells, though it seems probable that the fungus killed some of the bean cells.

Inoculation of leaves.—Pots of well-established seedlings of wheat, oats, rye, barley, corn, German and common millet, and sorghum were placed in a humid atmosphere (above 90% relative humidity) and atomized with suspension of H. No. 1 conidia. Well-defined spots occurred frequently on barley, less frequently on wheat. Leaf-spots due to a Helminthosporium, apparently H. No. 1, also occurred naturally on wheat in the greenhouse. Such spots were first pale; later with a mummified dark center surrounded by a pale zone; and were oval in outline. In rye the mycelium was seen to be abundant within the cells, and complete death

of the affected leaf, and also rotting without spotting, resulted. On the leaves in the humid air of the rag doll occasional spontaneous infections were noticed. In such cases the infection rapidly spread, involving nearly all of the leaf, which first turned pale, then very slightly brown. Aerial mycelium and conidia were profuse over the diseased portion.

SUMMARY CONCERNING ETIOLOGY OF FOOT-ROT

The evidence is conclusive that Helminthosporium is the cause of the basal rot of the wheat-stems. It is the only parasite constantly present, and has been repeatedly, and by many methods, proved capable of causing such rot. This conclusion is in accord with the findings of Beckwith (14), who as early as 1911 showed that Helminthosporium is a very common parasite within the tissue of wheat-plants. Bakke (6) in 1912 reported that when conidia of *H. teres* were placed on barley seeds, "At the end of two weeks' time there were not over seven seedlings to the row [originally there were twenty-five]. The roots were not in any sense indicative of a healthy state of growth." Oats and fescue-grass were not susceptible. A seedling blight of wheat observed since 1910 has been described by Stakman (113) in Minnesota, where in 1918-19 it became seriously injurious. The symptoms include dwarfing, foot-rot, and root-rot. The disease appears to be closely like, if not quite identical with, the one which is the subject of this paper. She proves conclusively that the cause is a Helminthosporium. A foot-rot of wheat due to a Helminthosporium having quite different morphological characters is also known in Sudan (see No. 46, page 184).

Certain of Ravn's experiments (91) conducted by inoculating seeds on wet filter-paper in a Petri dish, gave conditions much like those in the rag dolls. He makes no mention, however, of infection of the sheath nor of the occurrence of rotting of the basal region.

II. Evidence and Discussion of the Occurrence of Saltation within the Genus Helminthosporium

INTRODUCTORY

Early in my study of this Helminthosporium of foot-rot of wheat (herein designated as H. No. 1) it was noted that occasionally certain sectors of a colony growing on an agar plate differed more or less from the rest of the colony (Pl. XXII, 5; Pl. XXIII,1). This phenomenon is of rather common occurrence in work involving Petri-dish cultures of either fungi or bacteria, and little significance was at first attached to it; but later, when

the frequent recurrence of these variant sectors commanded attention, transfers were made from several of them to freshly poured agar-plates, and a transfer from the normal portion of the colony was added to each of these plates at a distance of about 2 cm. from the other transfer. The variant transfer was then marked on the bottom of the doubly occupied plate as M (indicating mutant), and the normal transfer as O (indicating original). In all the early transfers the M transfer resulted in a colony (M1) of decidedly slower growth and more profuse conidial production than that produced by the O transfer. The two colonies also differed markedly in general appearance owing to minute single differences which were often difficult to analyze, but which in the aggregate constituted distinctions which were so well-marked and obvious that at first sight one would say that the two colonies were those of two distinct fungi (Pl. XXIII, lower fig.; Pl. XXVII). When these colonies grew to fill, or nearly to fill, the plate, transfers from them were made to new agar plates, and later, transfers from these second plates, and so on, the series of transfers being a long one. It was found that the differences appearing in the M1 and O1 colonies were usually maintained on succeeding plates. These findings led to the tentative assumption that forms in the variant sectors were mutants or saltants of a more or less permanent nature, and a more serious study of this phenomenon was undertaken.

In their origin the variants or saltants always appear as sectors which differ from the portion of the parent colony adjacent to them (see Pl. XXII—XXV). To the naked eye the most common deviations from the original type are in density, color, and rate of growth. Closer observation, with the microscope, frequently shows variation in the grouping, size, and shape of the conidia, and in the branching of the mycelium. Quite often many small sectors of divergent character appear at the edge of a large colony, especially on a plate that is beginning to dry. Many of these divergencies are merely modifications due to local environmental changes, and whether they are more can be determined only by close study of their behavior in subsequent transfer or transfers. Closer consideration of the characters involved in these saltations is best deferred to the following topic.

In following this discussion it must be borne in mind that M refers to the variant sector on the plate on which it originated; M1, to the colony resulting from the first transfer from M; M-2, to that resulting from the first transfer from M1, and so on; and that O refers to the original colony in which the M arose. It is my custom to give the saltant a serial number (writing this on the plate in which it was found), and, usually, to transfer

both the saltant and the original to the same plate so that they may have the same environmental conditions, the identical quantity and quality of agar, and by growing close together may render comparison easy. Notes on the origin and subsequent behavior of the saltants were made under the serial number, and the transfers were designated by additional numbers. Thus, M98-7 refers to saltant No. 98, transfer 7.

Characters of Saltants as shown in Transfers

General appearance.—The colonies of the saltant and of the original when grown on the same plate were usually so strikingly different in general appearance (Pl. XXII, XXIII) that a mere glance sufficed to give the impression that they were colonies of two different species. This difference in general appearance is, on analysis, referable to one or more of the individual differences mentioned below.

Rate of linear growth.—Frequently the saltant was of much slower growth than the original, resulting in an M colony of much less diameter than that of the O colony, being often less than half of it (see two examples: one given in Pl. XXII and one in Pl. XXIII). In some instances, however, the M colony grew faster than the O colony.

Conidial production.—Frequently the M colony, especially when slow-growing, was much more productive of conidia than the O colony, so much so as to give the colony a decidedly perceptible darker color. In several instances, however, the M colony was of the opposite character, producing few conidia or, in some cases, going to the extreme of appearing to produce none at all. Generally speaking, rate of linear growth was in inverse ratio to that of conidia-production; while those saltants that were pale and possessed much aerial mycelium were usually of rapid linear growth and low conidial production.

Conidial clusters.—Some saltants varied strikingly from each other and from the originals in the mean number of conidia borne per conidiophore.

Conidial length, breadth, septation, and shape.—These characters, as evidenced by casual observation or by a study of graphs and the data derived from them, are shown to be strikingly different in various saltants. For clearness I present in this connection records concerning only a few saltants, giving graphs and data for others later.

Graphs of *conidial length* of saltants M35, M36, and M40, those which show greatest deviation from originals in this regard, are given in Fig. P with the essential data. It is to be observed that the modes of M35 and

M40 are 57.8 and 64.6 μ respectively, far below the mode of the original, which was 81.6 μ. M36 shows less striking difference, but this is still marked. Comparison of the means shows those of M36 and M40 to be approximately 17 and 18 divisions (1 division = 3.4 μ), while the original was 23 divisions. In other words, the conidia of M36 were only about three fourths the length of the normal conidium of H. No. 1. Such differences as they appeared in the microscope are shown in Pl. XXVI. The difference in variability is also strikingly large.

Striking variation in *conidial breadth*, both relative and absolute, was observed. Graphs and data of the more pronounced cases are presented in Fig. Q and others are given later. In connection with Fig. Y (Graphs 114-138) are given summary data on the conidial length of saltants included in this study. It is to be noted (Graph 6A, Fig. B) that whereas the mode of the ordinary conidium stood at 20.4 μ and no conidia exceeded a thickness of 23.8 μ, the modal thickness of M8-7 (Graph 75, Fig. Q) is 23.8 μ, with many conidia 27.2 μ in thickness, one even 30.6 μ. Such differences between saltants and the parental form are presented to the eye in Pl. XXVI.

The *ratio of conidial length to conidial breadth* is perhaps still more striking than the mere variation in length. In such variants as M6 (Pl. XXVI, *b*) and M8, while increased greatly in thickness the conidia were at the same time absolutely shorter, thus emphasizing to the eye both differences. The ratio of length to breadth in H. No. 1 is as follows:

$$\frac{\text{mean length}}{\text{mean breadth}} = \frac{22.62 \pm .05}{6.03 \pm .04} = 3.74 \pm .03^*$$

while in a sample of one of its saltants this ratio is

$$\frac{\text{mean length}}{\text{mean breadth}} = \frac{20.67 \pm .22}{7.82 \pm .11} = 2.64 \pm .04^*$$

and in another sample of the same saltant it is

$$\frac{\text{mean length}}{\text{mean breadth}} = \frac{19.58 \pm .30}{7.30 \pm .06} = 2.67 \pm .05^*$$

$$E = \pm\left(\frac{\sqrt{\left(\frac{Ba}{a}\right)^2 + b^2}}{A}\right)$$

*Probable error was computed according to the above formula kindly furnished me by Dr. J. A. Detlefsen, where a = probable error of A; b = probable error of B; and E = probable error of $\frac{B}{A}$.

Variations in *septation* were also noted; thus in Fig. R, Graphs 79-82 are quite different from Graphs 83 and 84, while all of these are lower than the results gotten from H. No. 1 in Graph 35, Fig. J. I attach but little value, however, to these variations because they seem inconstant.

Variation in *conidial shape* is common, some saltants showing the sides more nearly parallel than others, and the conidium as a whole less elliptical or fusiform.

Variation in submerged mycelium.—Aside from rate of growth and variation in branching which resulted in changes in density of the colony, differences in the submerged mycelium were observable in but two cases, most strikingly so in M26. In this saltant certain hyphal threads near the edge of the colony appeared to be much more vigorous than their neighbors, becoming a trifle thicker, and lengthening with such rapidity as far to outstrip the others, reaching out as single strands to a considerable distance beyond the usually even frontier of the colony, beginning then a dense, bushy branching in all directions, reminding one of witches'-brooms in trees. Numerous outposts of this kind give a peculiar lumpy appearance to the colony as seen by the naked eye. This peculiar mode of branching was clearly to be seen in M26, where it originated, and was frequent throughout subsequent transfers. Instead of single threads reaching out in this way, the mycelium sometimes formed fascicles which would grow out rapidly into new territory without branching, then suddenly branch profusely, forming a dense brush. These two rare characters were striking in effect both to the naked eye and under the microscope. Nearly every transfer from M26 or its descendants gave colonies with very strikingly marked sectors, characterized essentially by abundant conidial production, and therefore dark in color. The other sectors bore few conidia, were pale, and being a trifle less rapid in growth they were usually crowded out (Pl. XXX, lower fig.). These characters were maintained through many transfers. (See Pl. XXXI.)

Variation in aerial mycelium.—Saltant sectors and their progeny often differed from the originals in the abundance and character of the aerial mycelium. In some cases it was so scant as to be unnoticeable; in other cases so abundant and floccose as to obscure from vision the colony beneath. In character it varied from loose and fluffy to "ropy," the latter term indicating a tendency of many mycelial strands to twine together (Fig. 5, *c*, p. 104). In other cases it collected in clumps, the process being attended by peculiar distortions (Fig. 6, *e*). In some saltants these clumps were abundant and aggregated; in others few and scattered (Pl.

XXII, XXIII). The occurrence of clumps of mycelium upon the surface of the cultures has been stressed by Ravn (91) as of taxonomic importance (cf. also with Pl. XI, XIII, XXIII (below), XXVIII). H. No. 13, in one small sector, showed eight white clumps; the balance of the plate, none. In transfer M71 the clumping character seemed lost, but the following transfers were pale in type. In other cases the clumping habit seemed to be fixed and characteristic (Pl. XXVIII).

Variability in colony color.—The color is mainly due to abundance or scarcity of conidia or to abundance or lack of aerial mycelium, or to both. The white aerial mycelium is practically without conidia. Transfers from sectors with much white, sterile aerial mycelium were not always constant in these characters, but in many instances they were so; for example, M72, derived from single conidium C1-1, and M78, derived from M26. Differences quite comparable with these were noted by Crabill (36).

Zonation was well marked in some saltants and almost entirely lacking in others (Pl. IX, 3, 4, 5, 20). Some saltants formed *sclerotia* abundantly though the originals did not do so. *Density of colony* also differed, some saltants producing colonies of much denser growth than others.

Variability.—Variability itself was a distinctive character in certain instances. Thus, while the original of any given saltant is usually fairly constant in its characters and only occasionally gives rise to saltants, one saltant, M26 (Pl. XXX, below; XXXI), was definitely characterized by the fact of its inconstancy (see page 143).

Many saltants were tested as to their infecting power and their rotting power, but no real difference in these respects was noticeable between the different saltants, or between the saltants and H. No. 1. Since many species of Helminthosporium can infect many cereals this power may be rather fundamental in the genus and thus not be so readily subject to saltation as are less fundamental characters. I have no means as yet to measure slight differences in either virulence or rotting power. It may be mentioned here that Ravn (91) states that culture upon dead substrata diminishes the virulence of Helminthosporium. Whether such diminution was permanent or merely a temporary modification he did not determine. Edgerton (51) reports that different races of Glomerella differ in virulence.

Correlation of characters in saltation.—Certain correlations of characters are noticeable; thus, colonies of slow linear growth were usually high in

conidial production and *vice versa*. Correlations observed are indicated as follows:

Slow linear growth←→high conidia-production
Much aerial mycelium→low conidia-production
Pale colony←→rapid growth
Thickening of conidia→shortening of conidia
Pale colony→low conidia-production
Clumping of mycelium→low conidia-production

The differences in colony-color and growth-rapidity here noted, are much like those described by Edgerton (51) in Glomerella plus and minus strains. Crabill (36) notes also a correlation in that his minus strains were always of more rapid growth than the plus strains.

Tendencies in Saltation

Saltants showing very low conidia-production, verging on sterility, coupled with paleness of colony, occurred with the greatest frequency. A type with increased conidia-production and of slow growth was next in frequency. The latter of these types was the most frequently thrown during the early period of my work though it has been rare recently. On the other hand, the former type, which rarely appeared at first, is now the most common. A type characterized by thickness of conidium, as M6, M8, etc., has been frequent all the time. These three types were by far the most common, and may be said to show the three tendencies. Markedly short-conidia saltants were few, as were also clump-bearing types that possessed permanence. Strains that threw either of the two types first mentioned above were very likely to continue to throw similar types. The same may be said of clump-bearing types.

Stability of the Saltants

Many saltants have been tested in various ways to determine, to some degree, their constancy. Through numerous transfers on corn-meal agar the O colony and the M colony of many saltants have been carried side by side. Under such conditions, though the original may give rise to new saltations or the saltant may saltate further, the main portion of both the O and M colony, as a rule, maintains its characters.

It is manifestly impossible to test all the saltants to ascertain what their future behavior will be. All that can be done at present is to record certain observations concerning them. Several saltants possessing strongly distinctive characters have been repeatedly transferred and have maintained their characters through all of these transfers; and as far as can be foreseen

are as stable in their present form as are other fungi. Thus, saltants with short conidia (as M35 and M40) and saltants with broad conidia (M6 and M8) have been cultured and graphs of conidia repeatedly made, the saltant maintaining its character. For example, a determination of measurements of conidia of M35—made after several transfers and the lapse of some weeks—gave the following data:

M	σ	CV
17.31 ± .25	2.51 ± .17	14.50 ± 1.05

Comparison of the above data with data of Graph 65, Fig. P, shows that this saltant not only remains far below H. No. 1 in length but is also constant. It is particularly to be noted that all comparative conidial measurements were made under standard conditions. Other characters exhibited by saltants, such as color, zonation, and aerial mycelium, are similarly permanent when strongly marked. Saltants are, however, subject to further saltation and indeed in some instances are exceptionally liable to it, for example, M26. Not all suspected examples of saltation afforded by variant sectors proved to be permanent in character, and some lost their distinguishing marks after one or a few transfers. Such instability was not observed in cases of conidial length and breadth, or of pronounced pale colony-color, but was more commonly noted in cases of slight differences of aerial mycelium, slightly pale color of colony, clumping, etc. While all cultures were carried, for convenience, on corn-meal agar, and their differences were observed on this medium, all that were studied critically were passed through other media—autoclaved wheat-shoots and live-wheat—to determine whether such passage would alter the character of the saltant. The saltant characters were apparent on other media, as green-wheat agar or beef agar, though the general colony-character of both original and saltant was changed by the medium. After passage through these conditions, or through wheat, they were inoculated under standard conditions for all graphic comparisons. There is no evidence of alteration of the characters of the saltants by such procedure. In other words, the saltation is not a phenomenon associated with the medium and ended when the fungus gets back to its normal habitat.

Stability of the Saltants through the Conidia

Dilution platings of conidia of well-marked saltants gave colonies all alike and with all the characters of the saltant, showing permanence of these characters through the conidia.

Apparent Reversions

In several instances where colony color, aerial mycelium, or partial sterility was the saltant character, small sectors of the colony were so changed as to resemble closely the originals, and as far as tests were applied could not be distinguished from them (Pl. XXXII); in no case, however, where true saltant character was proved by constancy through several transfers did the whole stock revert; what appeared as reversion was limited to occasional sectors of the colony, and in no case did such change occur in the entire margin of a colony.

Suppositious Causes of the Variant Sectors

Several alternative suppositions other than that of saltation may be briefly discussed as possible causes of the variant sectors. The mycelium at a certain point may become weakened, or die, and the change in equilibrium resulting may cause the variant sector. Spores of another Helminthosporium or of some other organism may fall into the colony from the air, and the variant sector may represent merely a contamination. The inoculum used on a plate that shows saltation may have consisted of more than one strain or elementary species of Helminthosporium. The first supposition is open only to crude experimentation, while the second, if valid, implies a wonderful Helminthosporium-richness in the air of my laboratory as well as very faulty technique. Since saltation occurred after the fungus, H. No. 1, had been transferred many times by lifting a small bit of agar from the edge of a colony, the presumptive evidence that no mixture then existed is very strong. The following experiments bearing on these suggestions may, however, be worth recording.

Wounding.—A culture of H. No. 1 on corn-meal agar was allowed to grow to a diameter of about 4 cm. Then by means of a hot iridium wire the mycelium was killed at the points indicated in Pl. XXIX, above. In all cases the uninjured parts soon entirely outgrew the wounds, and the whole colony presented an entire, normal outer border with no evidences of saltation. In some instances a clear straight line extended from the point of wounding nearly to the edge of the colony. Evidently disturbance of equilibrium such as this did not cause saltation.

Mixed planting.—Acting on the knowledge that the saltants were frequently slow-growing, and thinking that possibly ordinary transfers might be mixtures of two or more races, of which the slower-growing one ordinarily remained masked, M8, a well-characterized saltant, was planted

on a corn-meal agar plate and allowed 24 hours to grow, by which time a vigorous mycelium had developed. A goodly quantity of conidia of H. No. 1 was then placed in the midst of this young but well-established M8 colony, but it remained uniform to full occupation of the plate, showing no saltations.

Implanting conidia of H. No. 1 in a partly developed colony of the same strain.—This experiment was conducted like that of wounding except that instead of using the hot wire conidia of H. No. 1 were implanted at the points indicated in Pl. XXIX, below. All implants within the colony grew sparingly and resulted in small clumps 1—2 mm. in diameter and highly sporiferous (Pl. XXX, above). Implants at the edge grew poorly, but those a few millimeters outside the colony became established and grew well, each implant developing as an independent colony and inhibiting advancement of the old colony, but bearing no resemblance to a saltant. In one case, however, such implants showed marked change in characters and are still under culture as saltants (M70, Pl. XXX, upper fig.), though efforts to produce other saltants in this manner were fruitless.

Implanting other Helminthosporiums.—In a way similar to that of the last experiment numerous other species or saltants (e.g. H. No. 2 and M6) were implanted in an H. No. 1 colony, and always with the result that the implant either failed utterly to establish itself or developed as an entirely independent colony that did not blend with the main colony, being in this unlike a saltant sector in character. If implants were put about 3 mm. beyond the tips of the advancing mycelium, the conidia were observed to germinate before the mycelium of the H. No. 1 colony arrived, but even such implants became entirely submerged and lost.

Two entirely distinct types of Helminthosporium, found intermingled on a single grain of wheat, were planted together—an oese of suspension of the mixed conidia—on an agar plate. The resulting colonies gave the two types of Helminthosporium, but did not give the sectors so characteristic of saltants.

Saltation not due to parasites.—The saltant sectors and their transfers often differed so strikingly from their originals, particularly when they bore few conidia and had much white aerial mycelium (see Pl. XXVII) as to suggest that perhaps the great difference was due to a parasite growing in the Helminthosporium colony. Close microscopic inspection of saltant sectors showed that there was only one type of mycelium present, that it was all indistinguishable from Helminthosporium mycelium, and

that no conidia indicating contamination were present, therefore, if the colony were parasitized it must be either by a mycelium like that of Helminthosporium and without conidia, or by some virus of unknown character. To test this possibility well-established colonies of H. No. 1 were inoculated with such striking saltants as M84. Transfers of M84 were also made to points near the circumference of the H. No. 1 colony. If M84 bore a parasite of any kind this parasite might be expected to invade and overgrow the H. No. 1 colony. This it did not do, but the two colonies halted a few millimeters apart in the manner characteristic of two Helminthosporium colonies. It is quite clear that the idea of colony parasitism is untenable in this connection.

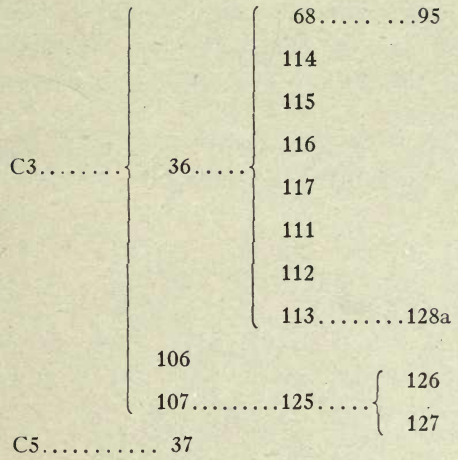
Position of inoculum.—Since it was possible that the differing appearances presented by the various sectors might be due to the position of the mycelial strands in or on the agar, that is, on top of it, in it, or below it, tests were made in three ways: 1, by placing conidia in an oese of water on the surface of poured agar; 2, by similarly placing conidia, without water, in a shallow scratch made in the agar; 3, by so cutting the agar that a flap about a square centimeter could be lifted and inoculated on the lower side, that is, the side in contact with the glass, the flap being then put back in place. These three modes of inoculation resulted in colonies of indistinguishable character.

Saltations from Single Conidia

Eight separate pure cultures were made from single conidia. The eight colonies were under careful microscopic control from the time of planting the conidia, through germination, and until the colony was well developed, and it is certain that in each instance the colony was from a single conidium. These pure strains, all alike in colony character, were labeled C1, C2, C3, etc. Well-marked saltants appeared in four of them as follows:

C1.....{ 72
110

C2.....{ 108
109.....{ 119
120
121.....122
128



Thus, it will be seen that single conidium 3 gave rise to sixteen clearly defined saltants; C5, to one; C1, to two; and C2, to seven—demonstrating absolutely that these saltants were not due to impurity of cultures. Evidently the saltant sectors do not result from contaminations.

Frequency of Saltation

It is impossible to give any mathematically accurate statement as to the frequency of saltation in Helminthosporium. One hundred and twenty-six variant sectors were selected, transferred, and more or less studied; and this number could easily have been doubled or trebled. It is not probable that all the forms in these sectors were truly saltants; doubtless some of them were mere modifications, but the number that were permanent in character is large. How many of these saltants agreed with each other in observable characters it is also impossible to say, but since they arose independently it may be that they do not often agree absolutely. The percentage of saltants, based on those theoretically possible, is, however, small even in races that are most actively saltating. Thus in a colony 6 cm. in diameter there are probably more than 5,000,000 cells, and theoretically it appears probable that saltation occurs in a single mycelial cell, or perhaps by the union of two cells, yet saltations occur with even less frequency than one to each 6-cm. colony, therefore less than once out of 5,000,000 possibilities. In this connection, though no direct comparison is possible, it may be noted that East (47) considers the occurrence of twelve inherent variations in observations made on 100,000 hills of more than 700 varieties of potatoes, that is, about 1:10,000, as an unexpectedly high rate

of frequency. In tobacco only one bud variant was noted in 200,000 plants. Benedict (16) regards the production of 50 new Boston ferns in fifteen years as rapid. East (46) notes that all of the asexual variations have been losses of characters.

The pedigrees of the various Helminthosporium saltants which I have studied are indicated in the following table:

PEDIGREE TABLE OF SALTANTS

H. No. 1...
- 3.....26*.....65
- 3.....26*.....59.....81
- 3.....26*.....59.....82
- 3.....26*.....53*, 56*, 57*, 58
- 3.....26*.....60, 61
- 3.....26*.....78
- 3.....26*.....89*
- 3.....26*.....90*
- 3.....26*.....96
- 4.....73.....76
- 5*.....9*.....69
- 5*.....9*.....92
- 5*.....9*.....93
- 5*.....9*.....94
- 2*.....88
- 2*.....88a.....88b
- 2*.....88a.....88c
- 2*.....88a.....88d
- 13.....71
- 15*.....64*
- 32.....79
- 35.....62
- 35.....63
- 40.....130.....132
- 40.....130.....131
- 40.....77
- 40.....122
- 40.....134
- 40.....135
- 49.....80
- 83.....118
- 83.....123
- 83.....124
- 1*, 6, 7, 8, 10, 12, 14, 16-19*, 20, 21*–25, 28–30*, 31*, 32*, 33*, 34*, 38*, 39, 41–48, 50, 51, 70*

H. No. 7...
- 52.....74
- 86
- 85
- 104
- 103

H. No. 8...
- 98
- 99
- 100

H. No. 9...
- 69
- 101
- 102

H. No. 13.....75

H. No. 14..
- 66
- 67
- 91

H. No. 17.....129

Unknown.....27.....
- 105
- 84
- 87

H. No. 34.....97

H. No. 39...Gave many saltants not numbered.

*Numbers followed by an asterisk are pictorially represented in the plates.

The apparent paucity of saltation in the strains other than H. No. 1 may be due in large part to the fact that these strains have been cultured to much less extent, though there is also evidence that H. No. 1 really is more actively saltating than are the other strains; indeed several strains, as H. No 2 and H. No. 29, have given no evidence of saltation. Saltation seemed to be as frequent in cultures derived from single conidia as from other cultures.

Saltations occurred on Various Media

Saltation was not confined to corn-meal agar, but was seen to occur also on green-wheat agar and on washed agar. The discrepancy in conidial measurements on two shoots (one in plate *e* and one in *e'*, cited on p. 121) may have been due to saltation on the washed agar. H. No. 34 as well as H. No. 1 showed saltation under standard conditions, that is, on washed agar on which wheat shoots were laid. Several of the shoots bore only sterile, white aerial mycelium, while the others were black owing to the usual number of conidia. Repeated transfers demonstrated the permanence of these characters.

Saltations and Modifications occurring in Test-tube Cultures

Certain cultures received from correspondents under the label Helminthosporium remained largely or quite devoid of conidia. The following are brief descriptions of such Helminthosporiums.

H. No. 11, of which graph of conidial length is given in Fig. S (cf. with Graph 42, Fig. K) and conidial-breadth in Graph 101, Fig. V, differed in general colony-characters from H. Nos. 1, 3, etc., but most markedly in that it remained for the most part without conidia. Conidial septation is given in Fig. T, Graph 87.

H. No. 12, which was received under the label "*H. gramineum* (?)" evidently had sometime borne conidia, but transfers to many media under many conditions gave me none in any case.

H. No. 17, also labeled *H. gramineum*, under standard conditions on wheat and corn usually produced mycelium with no conidia, though in one case one wheat shoot gave conidia, while five others in the same dish gave none. From this one shoot the conidial-length Graph 97 (Fig. U) was made.

It seems to me that the three cases just mentioned should be regarded as those of saltants which have outstripped their originals in the test-tube conditions, while the rare cases in which they do bear conidia, par-

ticularly in the case of H. No. 17, given above, are to be regarded as further saltation or as reversions. In all of these cases of sterile cultures the mycelium, so far as can be judged, is like that of Helminthosporium, but the colony-characters are altered in ways easily compatible with the changes following loss of conidia-producing power and consequent change in vegetative vigor. Ravn (91) mentions frequent sterility of cultures in connection with Helminthosporium.

H. No. 18, labeled *H. avenae*, gave conidia only once, on one wheat shoot, although numerous trials were made. Its case is almost exactly like that of H. No. 17. From the very few conidia, though inadequate, Graph 98 (Fig. U) was made.

H. No. 19, labeled "*H. gramineum*," rarely gave conidia under any conditions, though somewhat more freely than either of the two preceding numbers. (See Fig. U, Graph 99.)

H. Nos. 13 and 14, labeled *H. sativum*, and Nos. 15 and 16, labeled *H. teres*, are particularly interesting as showing variations in test-tube culture. H. No. 13 is a lineal descendant of H. No. 14 while H. No. 16 is a similar descendant of H. No. 15. Graphs of these four strains also are given in Fig. U (Nos. 93-96). It will be observed here that the differences between Nos. 15 and 16, which are separate cultures from the same original isolation, are greater than the differences between strains known to be quite distinct. Graphs of conidial breadth of H. Nos. 13, 14, 15, and 16 are given in Fig. V.

H. No. 20, labeled *H. teres*, appears to be an excellent example of saltation in test-tube culture. It was so markedly different from other cultures that during the several months in which I was ignorant of its origin I thought that here was a clear case of difference between the European and the American species. Subsequent word from Dr. Westerdijk advised me of the American origin of this strain, and that she had received it in 1914 from Bakke, isolated from barley. It thus seems that this culture from Dr. Westerdijk is a direct descendant of the culture on which Pammel, King, and Bakke based their description (90), and that it now differs markedly from that description as well as from a culture (H. No. 3) which I received from Dr. Bakke which was also taken from the same plots that gave the original culture and was regarded by Dr. Bakke as being identical with *H. sativum*. Among my notes made before I knew of the origin of the culture I find this memorandum: "H. No. 20 is quite distinct from all other forms in my cultures in its quite uniformly 6-septate conidium with its squarish middle cell. Its colony-characters

are also distinct." (See Pl. IX, No. 20.) Conidial length is given in Fig. U, Graph 100; breadth, in Fig. V, Graph 102; septation, in Fig. T, Graph 88. (See also photomicrographs of conidia in Pl. XXVI, *d*.) By comparing these graphs and data with those of H. No. 3 and H. No. 1 it will be seen that the conidia are quite short and a trifle thick. The coefficient of cylindricity—78 for subcylindrical conidia, 74 for the more elliptical ones—is the highest coefficient shown in any of my strains (cf. with page 119). It seems very probable, therefore, that either the culture was contaminated in Dr. Westerdijk's laboratory or the other culture in Dr. Bakke's hands; or that Dr. Bakke's description was erroneous or that one of the cultures was contaminated by me; or that the facts represent a real hereditary change in morphology during a long series of transfers—and I incline strongly to the last of these alternatives.

H. No. 23 showed what appears to be a modification rather than a saltation, in that in the original culture received from Miss Weniger there were many abnormal tri-pointed conidia (see page 101). It is suggested that a change somewhat like this, if permanent, may have given rise to the forms with unequal central cell, for example, to H. No. 29.

All of the above-mentioned examples appear to represent clear-cut cases of change in morphological character in test-tube culture. The only essential difference between these changes in test-tube culture and the saltation reported in Petri dishes is that in the cases of the Petri dish selection of the saltant was voluntary, while in making transfers from tube to tube the selection was accidental.

Saltations in Nature

That changes do occur under my culture conditions renders it highly probable that they also occur in nature—in the fields. Thus a saltant strain may become established on one wheat plant, form large numbers of conidia, gain foothold in a region, and then enlarge this foothold, perhaps to cover large areas. That one strain may thus outgrow another has been shown by Crabill (36) and is evident in my own work (Pl. XXX, XXXI, lower figs.). The fact that so many strains of Helminthosporium differing slightly but distinctly from each other, yet agreeing closely in general, can readily be isolated from cereals, indicates that probably this also has naturally happened, and that in the fields we have today large numbers of races or strains of closely related forms derived more or less recently from a common parent stock. To test this hypothesis experimentally, in field or greenhouse, by inoculation with pure cultures, and later isolating organ-

isms to search for differences, does not seem a promising line of research because negative evidence would be valueless, while positive evidence would be obtained only most rarely, even though saltation is very common. Since, even in my most rapidly saltating strains, changes occur in only 1 out of 5,000,000 cells, and re-isolations from soils would give colonies from single conidia only, that is, from a small group of cells, the evidence of saltation by this method of investigation could reasonably be expected only once in several thousand platings.

Notes concerning Selected Individual Saltants

Unless otherwise noted the permanence of the saltant characters were tested by repeated transfers. Colony-characters were determined on corn-meal agar; measurements of conidia and other conidial characters, under standard conditions.

M1. Origin slightly zonated (Pl. XXIII, 1), few conidia. M1-1 grew faster than its origin; ratio, 6.5:8; characters maintained through several transfers.

M6-1. Much like M1, but with decided difference in conidial breadth (Graph 70, Fig. Q).

M8. Growth slow; conidia few, pale, and thick (Fig. Q); septa few. The squarish cells very striking; differences apparent also on green-wheat agar.

M12-3. Quite distinct in septation and breadth. As to conidial length, see Graph 121, Fig. Y, and data.

M17-3. A distinct variant in thickness, septation, and shape. As to conidial length, see Graph 138, Fig. Y, and data.

M26. (See page 143, and Pl. XXX and XXXI.)

M35. Characterized by its very short conidia (see page 141 and Pl. XXVI, *c*).

M36. Derived from a single-conidium culture; conidia thick.

M38. The colony had much aerial mycelium and was quite white, though a shade of black from the surface-agar showed through (Pl. XXVII). It continued through many transfers as a pale form with scant conidia.

M53. (Pl. XXX). Very different from its origin, being covered with much loose, white, aerial mycelium, rendering the whole colony white and fluffy in appearance, while the original colony was neither white nor fluffy.

M54 and M55. These also were two white, woolly colonies.

M56 (origin, Pl. XXX) and M60. These were from a dark fast-growing sector of M26 and maintained character.

M57 (Pl. XXX, below)—M61 were from pale, fluffy, slow-growing sectors of M26. M61 eventually split up into many pale and dark sectors.

M62. The colony grew faster than its origin, and bore but few conidia.

M64 (Pl. XXVII). The colony was entirely white and fluffy from much aerial mycelium. It originated as a pale sector in M15-1.

M65 and M68. These were much like M62, and maintained character through many transfers.

M70. This arose where two colonies of H. No. 1 had been implanted just outside the edge of a colony of H. No. 1 (Pl. XXX, upper fig.). Both these colonies were strikingly different from the original, with much white and fluffy aerial mycelium Transfer from one of them showed the character permanent through several transfers (Pl. XXV), but whether this saltation was actually induced by the implanting or whether a saltant was unconsciously selected for implanting can not be told.

M72. Origin a most striking, fluffy, white, sterile sector. Characters transmitted.

M75. A non-clumpy sector from an original that bore many clumps. Grew faster than its original.

M78. Very loosely growing, stringy, white, few conidia. Character maintained, but the saltant soon threw many strongly contrasting light and dark sectors, which, however, did not show permanence after several transfers.

M79. Origin like M78, but character soon lost in transfers.

M80. Very fluffy and white and rapid-growing.

M81. Had a narrow (1 mm.) pink line parallel to the colony-edge and about 5 mm. from it. This line advanced, remaining narrow, as the colony grew.

M83. Originated on green-wheat agar as a black irregular sector of smooth surface, the remainder of the original having a fluffy surface.

M85 and M86. Two very fluffy white sectors. One of these maintained its individual character; the other did not.

M87. Whitish in its origin, and in succeeding transfers this character was intensified.

M88. Very fluffy and white; permanent through many transfers.

M88a. A pale sector, a transfer from which threw numerous variant sectors, some light, some dark.

M92. Originated in thin, sterile sectors (strands) which produced sclerotia at their outer ends. In transfers the colony bore many sclerotia.

M93. White and fluffy.

M94. Very pale and with many clumps.

M95. Rapid-growing, pale, with few conidia and many clumps. Several sectors later reverted to an appearance like that of the original.

M101—M105 were all fluffy, with white aerial mycelium.

The discussion of the causal fungus of foot-rot has so far been based, for simplicity, upon H. No. 1. Other strains of a Helminthosporium of the same general type have been isolated from cases of foot-rot from Madison county and have been proved capable of causing foot-rot. For example, in the spring of 1920 five isolations from one lot of material were made. These I designate as H. No. 1*a*, H. No. 1*b*, H. No. 1*c*, H. No. 1*d*, and H. No. 1*e*. All of these, in colony character and morphology, agree closely with H. No. 1, but H. No. 1*a*, *b*, *c*, *d*, have graphs of conidial length as shown in Fig. W, while H. No. 1*e* differed materially. It is obvious that the first four may be considered as of one strain, the last, *e*, of another strain, both strains differing somewhat from H. No. 1. The conidial breadth of H. No. 1*a* was as follows:

f	M	σ	CV
13	5.88 ± .07	0.39 ± .05	6.79 ± .89

Conidial septation of H. No. 1*a*, H. No. 1*b*, and H. No. 1*c*, is given in Figure X, Graphs 111-113.

General Discussion of Saltation

The existing differences in definition and usage of the term mutation, as also our very limited knowledge of cytological conditions in the genus Helminthosporium and our ignorance as to whether it has sexual stages, have led me to select the term saltation for the variations here discussed.

The term mutant is defined by Dobell (43), following Wolf (127) and Baur (11), as follows: "By *mutation*, accordingly, I mean a permanent change—however small it may be—which takes place in a bacterium and is then transmitted to subsequent generations. The word does not imply anything concerning the magnitude of the change, its suddenness, or the manner of its acquisition. The term denotes a change in genetic constitution. All other changes which are impermanent—depending generally upon changes of the environment—and not hereditarily fixed, are called *modifications*. The word 'mutation' has been used with such different meanings by so many bacteriologists and others, that the foregoing statement seems called for." Brierley (28) defines a mutation as "a genotypic change in a pure line"; and Vaughan (121), as "Those changes in form or

function which persist through one or more generations after the cause of the alteration has ceased to operate."

Since the variations herein reported occur in structures purely vegetative and result from no intervening sexual act, they are in kind comparable with vegetative variation known elsewhere—bud variation, etc.—with the exception that since the mycelium, consisting of a single row of cells, is the seat or origin of the variations the case is morphologically simpler than where tissues are involved, as in bud variation. De Vries (122) classes bud variations in general with mutations in that they appear as clear-cut discontinuous variations. Many examples of vegetative variation have been studied extensively and reported upon under the terms mutation, saltation, sporting, etc. Cramer (37) gives a very complete summary of the known cases in 1907. East (46), Stout (120), Ghys (61) and Shamel, Scott, and Pomeroy (102, 103) give reports, with extended bibliographies, concerning bud variation in the potato, Coleus, chrysanthemum, and lemon among the phanerogams. The transmission of such variations in the potato has been carefully studied by East (47, 48, 49) with the general conclusion that these asexually appearing variations concern characters that Mendelize in sexual reproduction. In the Pteridophytes, Benedict (16) reports saltation in the Boston fern. Dobell (43) summarizes the evidence from some twenty-eight papers concerning variability among the bacteria, discussing them in two categories: (1) physiological mutations, that is, changes in power to produce ferments or pigments; and (2) morphological mutations. He closes the first part of his discussion as follows: "It seems legitimate to conclude from the foregoing facts that some races of bacteria are able permanently to acquire new characters under certain conditions." He considers only two cases of morphological mutation, citing the work of Barber (7), who produced a race of long bacteria by single-cell selection, and of Revis (94), who claims to have produced a new race of *Bacillus coli* by the use of malachite green. Both Laurent (76) and Le Poutre (77) conclude that by passage a harmless bacterial species may acquire real virulence. An extensive résumé of the question of mutation in the bacteria is also given by Baerthlein (5). Numerous variations of yeasts, both morphological and physiological, are reported by Guilliermond (62).

The validity of the conclusion maintaining that there is mutation among the bacteria and yeasts has been attacked by Brierley (28) on the ground that the changes reported as mutations are merely due to segregation of organisms of aberrant type from an originally mixed popula-

tion. The very large mass of corroborating positive evidence, though really conclusive only when based on single-organism cultures, makes it extremely probable, to say the least, that such saltations or mutations do occur in these groups.

Among the Eumycetes several examples occur of what appears to be saltation. Edgerton (50) in 1908, writing of *Glomerella rufomaculans*, states that "of the more than thirty collections studied from over twenty hosts, with less than a half-dozen exceptions all gave at least slightly different characters. Even the two collections on apples from Missouri and Illinois did not give exactly the same characters, but the differences were slight. The two collections from apples in the north, however, gave entirely distinct characters from the more southern forms on the same host. The southern form, especially on sugar medium, was characterized by very rapid growth and a very dark greenish-black color of the substratum and aerial hyphae; while the northern form grew more slowly and had very little dark color. Generally in the latter the aerial hyphae were colored pink from the profuse development of conidia. Even the form on quince collected in New York did not give the same characters as the northern form on apple. The forms on orchid, Coffea, and Sarracenia, collected in the same greenhouse at the same time, were not exactly alike in culture media."

Other examples were given by Edgerton (50) in 1908 of what appears to be the same phenomenon as that under discussion here, but as there is no evidence that he worked with single-conidia cultures* his apparent variations may have been due—though it is highly improbable—to segregation of elementary species. His general conclusion follows: "The only explanation of the phenomenon is that one or more individuals of the original form changed quite suddenly their course of development under cultural conditions. It is undoubtedly a Gloeosporium of the Glomerella type, with the development of the perithecia considerably different from other known forms. Mutations, so far as is known by the writer, have not previously been recorded among fungi, but the form just described seems to be one without question."

Here, too, should be mentioned the plus and minus strains of Glomerella studied and reported on by Edgerton (51) from single-conidia cultures, though this may represent a differentiation of sexes rather than of races. He gives several citations which indicate that other workers have

*In a personal letter dated February 7, 1921, he writes regarding this as follows: "All of the cultures that I used in that work were obtained by the dilution plate method and presumably came from single spores."

studied these strains, and records the belief that the culture mentioned above "as a possible mutation . . . . was really the minus strain of the bitter-rot fungus."

In 1909 I reported with Dr. Hall (Stevens and Hall, 118) for the genus Ascochyta variant sectors in Petri-dish cultures quite like those reported in this paper; but since the study was not made from single-conidium isolations it is possible, though not probable, that I had merely a segregation of elementary species.

Shear and Wood (105) in 1913 reported that in cultures of Glomerella started from a single ascospore "an important variation or mutation suddenly occurred in the fourth generation and was transmitted through three following generations." They cite other variations, less permanent, which they regard as fluctuations.

Burgeff (31) in 1914 reported results from an extensive study of asexual variation in the genus Phycomyces. Working from single-spore isolations he got great diversity in many characters.

Crabill (36) in 1915 described two strains of *Coniothyrium pirinum* which he designated as plus and minus strains that differ markedly in several characters, particularly in size and abundance of pycnidia (verging on complete sterility), and in color of colony. He says:

"The cultural studies show that minus strains may arise from plus strains by a sudden sporting or mutation. An objection might be raised that these cultures were impure, i. e., mixtures of two strains. In anticipation of such an idea it seems desirable to state that frequent pourings of dilution cultures were used to preclude such a possibility. Progeny were then selected only from well-isolated plant, microscopic examination of which showed that each was derived from a single spore. . . . . Both strains have repeatedly arisen from the progeny of a single plus spore. When once purified the minus strain remains constant from generation to generation. The variation is apparently occurring in only one direction. . . . . The only explanation which remains is that the minus strain is a sport or mutant arising from the plus strain at irregular and unprognosticable intervals."

The plus strain by sudden sporting gives rise to the minus strain, but minus strains were not seen to give rise to plus strains, that is to say, the saltation is orthogenetic. He states also that the variation apparently occurs in the spore and not in the mycelium, which is quite the contrary to my findings. He finds, in agreement with my work, also with Kleb's law (74), that the minus strain, that is the sporiferous one, grows faster

than the plus strain. The sectoring and the colony differences which he pictures are much like those shown for Helminthosporium in this paper. He also adduces evidence to show that these plus and minus strains occur in nature and have been isolated by independent workers. He attempted in four ways to induce saltation artificially but met with no success. Crabill (35) has reported, in abstract, "a somewhat similar mutation in a fungus belonging apparently to the genus Phyllosticta." Blakeslee (21) reports: ". . . in 1912-13 I found numerous variants of various degrees of distinctness in the offspring of a single plant (Mucor) obtained by sowing non-sexual spores." Writing of *Mucor genevensis* he says (20): "In all, somewhat over 38,000 colonies from individual sporangiophores have been inspected and a relatively large number of variants of different degrees of distinctions have been obtained . . . . the mutants tend eventually to revert to the normal type. Two, however, have seemed more stable." He concludes: "They add to the evidence, already obtained from other groups, that mutations are not restricted to processes involved in sexual reproduction."

Brierley (27, 28) reported an albino *Botrytis cinerea* which was a form with pale sclerotia though the parent form always had black sclerotia. This albino was observed to arise from a colony derived from a single conidium and from a race that had been under culture for considerable time, always producing black sclerotia. The purity of his culture seems to have been carefully guarded, and this case, though standing alone, would furnish positive evidence of the sudden occurrence of a hereditary difference in this fungus.

Dastur (40) in 1920 described saltations in *Gloeosporium piperatum* consisting in the absence or presence of perithecia, acervuli, or setae, and in the development of aerial mycelium. He says: "Thus all of a sudden the original sterile culture broke up into two different strains, one producing only perithecia on sterilized chilli stems and the other forming acervuli with and without setae." Some of these strains were not constant in character, but others persisted through many transfers. He states that great or sudden variations have never been observed from conidial strains, but that "in cultures made from perithecia of the strain of *Gloeosporium piperatum* incredibly large and often very sudden variations have been obtained." Burger (32) in 1921 reported mutation of several types in Colletotrichum, involving permanent changes in many characters. He found these occurring in cultures derived from single spores and showed that they were permanent through the spores. Jennings (70), who worked

with the shelled rhizopod Difflugia, states that most of the work on uniparental reproduction has yielded the result that during such reproduction the hereditary constitution (genotype) appears not to change though the organism may differ much in outward character. Many papers cited support this view though some are opposed. Jennings says with regard to his own results: "After many generations of descent from a single progenitor, such a single family . . . . has differentiated into many hereditarily diverse stocks." These diverse stocks differ hereditarily not only with respect to particular single characters but also with respect to the combination of characters. Many individuals of uniparental reproduction have shown a marked permanence of hereditary character in single lines of descent, all the progeny being like the parent in hereditary constitution; and further, many such lines, diverse in hereditary constitution may exist in a population, and the effects of selection consist mainly, if not entirely, in the isolation of such diverse lines.

East sees no reason to believe bud variation different from germinal mutation and says that it may be progressive, digressive, or retrogressive. Bateson (10) holds that bud variations are due to qualitative cell-division in somatic tissues, giving somatic segregation of unit factors. East points out that in the large majority of cases of bud variation there has been simply the loss of a *dominant* character and hence the appearance of a related recessive character. In some cases there is absolute disappearance of the dominant character; in other cases it appears to be latent, and it may reappear. Variations in color constitute over 70% of all bud variation. Colony color in Helminthosporium is also a very common variable but this is, in all probability, entirely incomparable with color variation in flowering plants.

Brierley (28) holds, for Botrytis, that even if sexuality occurs, the fungus is "on all evidential criteria, an asexual homozygotic organism in which the isolation of a single spore strain necessarily implies the isolation of a 'pure line' ". . . . . A genotypic change in a pure line is a mutation." Similarly, Shear and Wood (105) regard individuals originating from single spores of Glomerella as homozygous, though on reasoning differing somewhat from that of Brierley. Crabill (36) also holds that since his fungus "reproduces asexually segregation from heterozygous parents cannot explain the origin of the strains." Accepting Brierley's criteria, my Helminthosporium single-spore isolations are equally homozygotic. In at least three forms on cereals Helminthosporium is known to be the conidial stage of the ascigerous genus Pleospora. As ascigerous

stages are known in many cases to arise sexually, it may be expected that all perithecia represent sexual stages and a sexual act. The particular forms with which I am working, that is H. No. 1 and derivatives, have given no evidence of perithecial formation nor is it actually known that they possess sexual stages. The presumption, however, is that they do, so it is quite possible that my culture of H. No. 1 is derived from an ascospore, that is, may be the result of sexual parentage; and this sexual act may have occurred in the not distant past. This is all hypothetical but it appears to me to point to a possibility of an heterozygotic condition in Helminthosporium, as well as in Glomerella as reported by Shear and Wood (105) and in Coniothyrium as reported by Crabill (35), since so few generations may have elapsed since fertilization that the heterozygosis has not yet been eliminated. Such supposition in the case of Botrytis is less tenable.

It is suggestive to note here that Dastur (40) found variation a common phenomenon only in strains that were recently derived from perithecia; also that bud variation is more common in hybrids (East, 46). It is therefore thinkable that such of my strains of Helminthosporium as are saltating are of recent ascigerous origin, while others that are not saltating (for example *H. ravenelii* and *H. geniculatum*) are of distant ascigerous origin.

If heterozygosis be eliminated from the discussion two other possible explanations, suggested by Brierley regarding Botrytis, may be considered here, namely, that of nuclear transference during mycelial anastomosis (Fig. 5, p. 104) or that of cytoplasmic contamination by such anastomosis. Evidence on these questions, both from cytology of the mycelium and from knowledge of sexuality, is quite lacking. Accepting none of the above hypotheses, the saltation would be a mutation in the strictest sense of the term.

Reported mutations in Aspergillus and Penicillium described by Arcichovskij (2), Waterman (125), and Schiemann (100), and said to be induced by environmental changes, are quite extensively discussed by Brierley (28), who, repeating much of their work, concludes that when the fungi showing these changes are returned to their original environment they resume their original aspect; that in fact the changes were mere modifications due to environment.

The cases reported by Brierley, Burgeff, Blakeslee, and Crabill, and my own work reported herein, all based on single-spore culture and carried under observation for sufficient time to give assurance of permanence, constitute complete proof of the occurrence of the phenomenon of sud-

den change in character among the fungi. The evidence from Edgerton, Shear and Wood, and Dastur, while not so complete, is strong collaterally. It would seem from all this evidence that this phenomenon is common and widely distributed among the fungi, though unquestionably it is more common in some species and races than in others.

Taxonomy

The classification of these Helminthosporium "forms," indeed of all the fungi imperfecti, presents unusual difficulties. That they are only "forms" of which we do not yet know the "perfect" stage, is no more relief from the necessity of classification than is incomplete knowledge adequate reason for delay in attempts to classify other plants. In the present instance some well-defined "species," in the old sense of the word, stand out—for example *H. ravenelii*—while, on the other hand, several of the strains of Helminthosporium in my collection differ in one or more slight ways yet agree with each other closely in general type. For example, my H. No. 1, H. No. 11 (isolated by Stakman from wheat in Minnesota), H. No. 13, isolated by Durrell in Iowa, H. No. 23, isolated by Weniger in North Dakota, and an unnumbered one isolated by Hoffer in Indiana, are all clearly the same general type of organism, and yet they differ from each other in minor particulars.

It is evident that we have in the genus Helminthosporium large numbers of races that vary consistently and constantly, though but slightly, from each other. These variations may be morphological in the usual sense of the term, or as shown in cultures, or as demonstrated biometrically. It is quite probable that here, too, there are, as elsewhere in the fungi, differences in virulence, and therefore in biologic relationship, and physiologically. Examples are numerous among the fungi where such comparatively minor differences are regarded as of specific rank and the new group is designated by a new binomial. There are also numerous examples where such slightly variant types are regarded as varieties or races of the species. These varieties or races have been variously designated as follows (or by the equivalents of these terms in other languages):

Physiological species (Hitchcock and Carleton—67)
Species sorores (Schroeter—101)
Biologische Spezies (Klebahn—73)
Biologiske Arten (Rostrup—95)
Schwester Arten (Schroeter)
Biologische Rassen (Rostrup—95, 96)
Specialisirte Formen (Eriksson)

Formae speciales (Eriksson—55)
Gewohnheitsrassen (Magnus—80, 81)
Races specialiées (Marchal)
Mikrospecies
Biotypes
Elementary species (de Vries)
Pure lines (Johannsen)
Biological forms
Biological races

Fischer (56) adopts the practice of recognizing as distinct all forms which differ in their choice of hosts in so far as the hosts belong to different genera; a procedure that leaves the specific rank and name of the parasite subject to vicissitudes arising from subsequent changes in the conception of the taxonomy of the host. It is yearly becoming more evident that distinctions such as these are common in the fungi within what were previously regarded as groups of specific rank.

Biologic specialization in the rusts was announced in 1894 by Eriksson (55), and has since been abundantly attested by Stakman (109), Stakman and Piemeisel (111), Stakman, Piemeisel, and Levine (112), by Arthur (3, 4), and by others (57, 68). Abundant evidence that it occurs in the powdery mildews is afforded by Neger (85), Salmon (98), and Reed (92).

The first demonstrated cases in the fungi imperfecti were probably in Helminthosporium, reported by Ravn (91). It was demonstrated in Septoria by Beach (12). Reed (93), summarizing regarding biologic specialization, cites papers to show its occurrence in the following genera: Synchytrium, Albugo, Peronospora, Taphrina, Claviceps, Dibotryon, Rhytisma, and Colletotrichum.

Evidence that there is differentiation morphologically, slight but measurable and constant, has been found among the rusts by Arthur (3, 4) who, writing of Uromyces on Spartina, says that "the four races of this species exhibit not only physiological specialization but a certain amount of morphological differentiation." Similar findings are reported by Bisby (17) concerning *Puccinia epilobii-tetragoni*, by Stakman and Piemeisel (111) regarding *Puccinia graminis*, and by Arthur (3, 4) regarding *Dicaeoma poculiformis* on Phleum. Brierley (26) has demonstrated by single-spore cultures the existence of elementary species, morphologically distinct, within the species of Botrytis, Penicillium, and Stysanus. Gäumann (60) has shown *Peronospora parasitica* to consist of very numerous races separable on both biologic and morphologic grounds. Similar findings regarding Plasmopara are reported by Wartenweiler (124). *Pes-*

talozzia guepini was reported by Bartlett and La Rue (unpublished paper) to consist of numerous distinct strains. *Ascochyta chrysanthemi* was shown to consist of at least two distinct strains by Stevens and Hall (118). Crabill (34) states that there are four distinctly different types of *Phyllosticta pirina*, and that *Coniothyrium pirinum* (34) is similarly composed of distinct races. Burger (32) demonstrates a similar condition in the genus Colletotrichum. Wiedemann (126) has published species of Penicillium based on differences shown on culture media—a procedure very common in dealing with the bacteria. The wide-spread occurrence of slightly but constantly differing varieties within the species of fungi is apparent; also that two diametrically opposed methods of procedure are in vogue to meet the situation. One method gives specific rank to each elementary species, or race, or strain; the other restricts binomial designation to the larger groups (the collective species), recognizing that the latter consist of numerous smaller groups—the elementary species (or races or strains). Lotsy (79) suggests that the terms "Linneon"—defined as "a total of individuals which resemble one another more than they do any other individuals"—and the term "Jordanon"—for the elementary species—be employed. This suggestion, in that it recognizes the elementary species as properly in one category, and the group of elementary species as belonging in a larger category, both subordinate to the genus, is in accord with the general discussion of "Aspects of the Species Question" (29)—see particularly conclusions of Britton and remarks by Coulter. The difficulties regarding elementary species that beset the taxonomist in dealing with the flowering plants are manifoldly increased when the classification of the fungi imperfecti is in question. Thus in the genus Septoria there are more than 1200 named species usually delimited from each other by barely three or four characters, and these extremely variable. Many other genera present conditions equally bewildering. The result is that it is absolutely impossible, even with the type specimens in hand (and they are usually unobtainable), to determine species accurately. It is highly probable that many of the forms now listed as species in the fungi imperfecti are either identical or merely biologic races —that is elementary species. To designate each elementary species either in the fungi imperfecti or elsewhere by a binomial defeats the very purpose of the name and renders it not only useless but cumbersome. The conceptions of Lotsy and of Britton and Coulter as noted above, seem particularly applicable here and indicate the advisability of using a binomial to designate a group which shall comprise many elementary

species—a course that I have already followed in the case of Colletotrichum (115) and which was followed by Elliott (53) in dealing with Alternaria.

My H. No. 1, H. No. 1*a*, H. No. 1*b*, H. No. 1*c*, and H. No. 1*d*, the cause of foot-rot in Madison county, Ill., as well as my Helminthosporium numbers 3-9, 11-19, 22-27, 34, 37, 38, 42, and 43, all belong to the same general type and are characterized by a conidium that tapers toward each end from a point of greatest thickness which is nearer to the base than to the apex of the conidium. The conidia are therefore not typically cylindrical or even subcylindrical. While all of these numbers agree in general type, many of them differ somewhat from others in the collection. Thus Nos. 1 and 3 differ as is shown in Plates IX, XI-XIII, and, so far as observed, in this character only, and only under the conditions described. Others differ in modal spore-length or septation, in distinctness of zonation, or in other minor colony-characters (Pl. IX). Number 13 differs slightly even from No. 14, though both are derivatives from the same original culture; and the same may be said of Nos. 15 and 16. These differences which now actually exist, are probably due to unconscious selection of saltants in transferring from tube to tube. All of the numbers listed above which show constant, though but slight, differences from other numbers I regard as elementary species, the Jordanons of Lotsy. They need not be further characterized or differentiated than has been done in previous pages. One of these elementary species, H. No. 3, is derived from an Iowa culture probably identical in character with that from which Pammel, King, and Bakke described *H. sativum*, and this culture, No. 3, still agrees essentially with their description. All of this group of elementary species may therefore be regarded as belonging to the *Helminthosporium sativum* group, or Linneon. The question of the possible identity of this group with the *H. teres* and *H. sorokinianum* groups I shall not now discuss further than to point out that so far as can be judged from the picture of *H. sorokinianum* given by Sorokin (107) that species is not characterized by longitudinally eccentric conidia; also that subsequent to the publication of the species *H. sativum*, Bakke (6) states that "cultural experiments have determined that the disease is due to *Helminthosporium teres* Sacc." He adds: "He [Dr. Ravn] further substantiated my opinion that the disease was due to *H. teres* and similar to what had been so prevalent in Denmark during the years 1898 and 1899." Bakke, in a foot-note, however, adds: "A. G. Johnson, of Madison, Wisconsin, considers *H. sativum* and *H. teres* distinct forms." Saccardo, who examined *H. sativum*, sent to him by Bakke, expressed the

opinion that the disease was due to *Helminthosporium teres*. It may be remarked here that Ravn (91) says that leaves are the only substrata on which conidia are developed; which is certainly a marked distinction from the *H. sativum* group which sporulate so freely on agar of many kinds.

There is no question whatever in my mind that by means of biometry and a study of biologic relations and cultural characters, tenable distinctive diagnoses can be drawn up for many races of Helminthosporium on the five leading cereals. How many of these should be designated by binomials and how many left unnamed appears, on final analysis, to be a question of the utility of such naming, which, in turn, may hinge upon their economic or other importance rather than upon the magnitude of their morphological or other differences.

H. No. 20 is particularly interesting in that it is—if no error exists in its history—an example of saltation so great as to remove the organism entirely from the group under discussion (the forms with tapering conidia), and consequently to place it in a group (Linneon) different from that to which its known relatives (H. Nos. 13 and 14) belong.

Conclusion

The present study was undertaken with two leading objects: (1) to determine the efficient cause of the rotting at the lower part of the wheat stem; and (2) to throw light on questions of morphology and parasitology in the genus Helminthosporium. The questions arising from saltation injected an additional interesting series of observations. The evidence is complete that Helminthosporium can and does cause foot-rot at the base of wheat stems. The study has also shown the Helminthosporium (H. No. 1) to be a root parasite. This phase of the disease has been studied only incidentally, but it is worthy of searching investigation since it may lead to the rosetting often associated with foot-rot, and thus predispose the plant to foot-rot.

Summary

1. In the rotting base of the wheat a Helminthosporium is the only organism constantly present (p. 124).

2. The culture characters of this fungus were studied on many media (p. 79) and under many and various environmental conditions. Slight changes of nutriment, as afforded by small differences in agar formulae or by the temperature at which the agar was made, produced marked effect on growth-characters. Of many agars tried, corn-meal agar proved most useful. Cereal shoots autoclaved, served as a still more favorable medium.

3. Morphological characters are largely altered by environment. Quantity as well as quality of food produces change in characters. Humidity has an important influence on the production of conidia, on the aerial mycelium, and on sclerotial formation (p. 93), influencing even conidia length (p. 95).

4. The optimum temperature for growth is about 25° (p. 98).

5. Carbohydrates in the medium favor production of a dark color (p. 100).

6. Marked effect of nutrition conditions on conidial length, septation, and shape was noted.

7. From the above findings it follows that collections to be comparable must be made under similar conditions as regards the factors mentioned (p. 102).

8. A procedure to secure standard conditions for study of the fungus was devised (p. 180).

9. The mycelium, aerial and submerged, is described. The cells bear several nuclei. The senescent mycelium undergoes autodigestion (p. 108).

10. Conidia show distinct basal and apical markings. The wall is in two layers: the outer (episporium), thin and brittle; the inner (endosporium), thick and gelatinous (p. 111).

11. Germination is usually terminal; anastomosis of germ-tubes is common (p. 115).

12. The conidia are thickest at a point between the base of the conidium and its middle point. The concepts "coefficient of longitudinal eccentricity" and "coefficient of cylindricity" are introduced for purposes of more accurate description (pp. 117-120).

13. Conidial length, breadth, and septation are studied biometrically.

14. For comparison, a biometric study was made of *H. ravenelii* (p. 121).

15. The etiological relation of the Helminthosporium (H. No. 1) to foot-rot was demonstrated by its constant presence, by the absence of other parasites, and by its proved ability to cause infection and rotting under various conditions, as by inoculation of seedlings in Petri dishes, in rag doll, and in soil (pp. 124-128). The fungus was shown to enter cells of leaf-sheath, stem, and root.

16. A study of the infection phenomena shows important changes in the cell-walls of the host; and the development of appresoria and a callus-like formation (p. 128).

17. Many strains of Helminthosporium, some very different morphologically from others, can produce some or all of the phenomena of infection (p. 136).

18. Alternaria produces some of the marks of infection, including the changes in the host-cell, the "callus," and entrance into the host-cell. Penicillium can not do this (p. 137).

19. Wheat, corn, barley, rye, sorghum, Sudan-grass, and millet are more or less susceptible to rot by Helminthosporium.

20. Saltation, possibly mutation, is common in certain races of Helminthosporium (p. 139).

21. Saltation is evidenced in general colony-character; rate of growth; conidial production; conidial clusters; conidial length, breadth, septation, and shape; mycelial characters, color, zonation, and sclerotial formation (pp. 141-144).

22. Certain saltants differed so markedly from their parent as to far exceed the usually accepted specific limits (p. 141).

23. Certain correlations and tendencies of characters in saltation were noted (pp. 144-145).

24. The saltants were, in the main, permanent in character (p. 145).

25. They were permanent through the conidia (p. 146).

26. What appeared to be reversions sometimes occurred (p. 147).

27. Efforts to produce saltation artificially failed (pp. 147-148).

28. The saltation was not due to mixed plantings, and can not be induced by implanting or wounding (pp. 147, 148).

29. Saltations are not due to parasites (p. 148).

30. Saltations in abundance were derived from single-conidium cultures (p. 149).

31. Saltation is very frequent as compared with bud-variation noted on potatoes and tobacco (pp. 150-151).

32. Numerous variations in test-tube cultures are reported as probable examples of saltations (p. 152).

33. The Helminthosporium that causes wheat foot-rot belongs to the *H. sativum* group, which consists of a large number of elementary species (p. 167).

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*My notes from this important article were made from a photographic reproduction of a typewritten translation kindly loaned to the University by the Library of the U. S. Department of Agric ulture.

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APPENDIX

Methods

For measuring conidia.—The following procedure was found convenient. An ordinary bacteriological iridium wire was plunged into vaseline and then so laid across a microscope slide as to leave on it two complete, narrow, thin streaks of vaseline about 6 mm. apart. A small drop of water was then placed between these two vaseline lines and the conidia-sample added and evenly distributed. When the cover-glass was placed the vaseline prevented the conidia from scattering, and rendered it possible by means of the mechanical stage to measure every conidium, thus securing a more representative sample than would be the case if some conidia, perhaps of some particular class, were allowed to float away.

In sampling from standard cultures for purpose of conidia-measurement, a portion of a shoot about 6 mm. long that was evenly and densely covered with conidia was removed to the slide. Shoots were all evenly and abundantly sporiferous except in cases where entire shoots or parts of shoots were paler and bore more aerial mycelium.

To avoid unconscious selection in measuring conidia a mechanical stage was used, and all conidia encountered in certain predetermined positions in the field of vision were measured. Length was measured from extreme tip to extreme base; breadth, at the thickest point. Measurements falling exactly between two classes were temporarily so recorded, and later distributed equally between the two adjacent classes.

Measurements for coefficients are easily made by projecting the outline of the conidium, by means of a camera, upon quarter-section paper of convenient ruling. The paper may readily be oriented with the conidium in any desired relation.

The rag doll for inoculations.—An adaptation of the rag-doll seed-tests was found useful in inoculations. The doll was made of a strip of cloth, 6×50 cm. which was rolled to a cylinder about 6×2.5 cm. and placed in a test-tube 2.5×25 cm. with water, and autoclaved. In use the roll was removed to a sterile Petri-dish 17 cm. in diameter, the water removed to the desired degree by wringing, and the doll unrolled by the use of sterile forceps (Pl. XXXIII). Seedlings raised aseptically were

then laid on the unrolled doll and inoculation made in the desired manner. The doll was then rolled up, inclosing the seedlings, and placed again in the test-tube. For purposes of root inoculation the doll was suspended in the test-tube about five centimeters from its bottom.

Inoculations in soil.—In addition to the usual pot and bench inoculations, it was found convenient to use wide-mouth vials, 12×70 mm. (see page 128), which were lined with stiff paper (so cut as to open easily, Pl. XXXIII), filled with soil, and autoclaved. The paper envelope with the enclosed soil could readily be withdrawn from the vial, and opened in order to insert the seed, seedling, or inoculum, and later repeated examinations could be made without greatly disturbing the plant.

Imbedding conidia.—Conidia were raised under standard conditions (see next paragraph) and the entire shoot bearing them, together with the adjacent agar—a strip about 4 mm. wide—was removed to chrome-acetic killing-fluid, and imbedded in the usual way.

Procedure to secure standard conditions.—Petri dishes of 12 c.c. washed agar, when solid, were inoculated in the center with the desired organism. When, in the course of a few days, this had attained a colony-diameter of 2 to 3 cm., wheat shoots, autoclaved in water, were laid on the surface of the agar, the basal ends of the shoots touching the edge of the advancing colony. Usually about six shoots were used per plate, resulting in ample material. Aseptic wheat shoots were secured by the method described in the next paragraph. The shoots were cut for autoclaving when they were about 2-3 cm. in length. This medium was selected as being of appropriate composition and only very slightly variable. The washed agar in uniform quantity in Petri dishes of the same depth gave a uniform humidity, while the mode of inoculation was also uniform, doing away with many errors that arise when the quantity of the inoculum is a variable factor.

Growing aseptic seedlings.—Seeds were treated three hours in 20% fresh Javelle water, rinsed with sterile distilled water, and germinated on damp filter-paper in moist chambers (44). In the latter part of the study para-toluene-sodium-sulphochloramide was substituted for Javelle water in seed-disinfection. It was used in 0.5% aqueous solution, the seeds being immersed for twenty minutes. Such preliminary tests as we have made, indicate that a solution of 0.25 to 0.5% is efficient as a fungicide, while such solutions may be safely used without injury to the grain. It certainly possesses value for such uses in the laboratory, and may be of service as a fungicide in other connections. A rather

extensive test of its utility against the cereal smuts is now in progress. Para - toluene - sodium - sulphochloramide is a chemical of the formula (p)$CH_3C_6H_4SO(NCl)ONa$. My supply was secured from the Abbott Laboratories in Chicago, where it is manufactured.

Preparation of potato plugs.—Instead of the ordinary potato plug it was found useful to place a glass slip in a test-tube as in Fig. 2, p. 94, then to crowd into the tube a potato slice, bringing it into contact with the glass slip, and, at its angles, with the walls of the test-tube. This gave, in addition to the usual surface for observation, the places where the potato made contact with the glass slip and with the walls of the test-tube.

A device for the study of humidity.—To study the effect of changes of humidity on conidiophores and conidia, test-tubes were fitted with glass slips, and sterile wheat shoots laid across them (Fig. 2, p.94). The region 1 is of about 90% relative humidity; that of region 2 is below 90%. Combustion boats full of agar were used (Fig. 2) to secure high humidity for the whole culture.

List of Helminthosporiums used for Purposes of Comparison

H. No. 1. Isolated by F. L. Stevens May 18, 1918, from wheat diseased with foot-rot, from Madison Co., Ill.

H. No. 2. *H. ravenelii*, isolated by F. L. Stevens Jan. 17, 1920, from specimen received from A. B. Seymour, collected at Lake Charles, La., Oct., 1919, by E. E. Barnes. This specimen was thoroughly typical, and no doubt as to the determination can be entertained.

H. No. 3., labeled *H. teres*. Received from A. L. Bakke Jan. 5, 1920. Culture isolated Jan. 9, 1911.

H. No. 4. Isolated by F. L. Stevens Jan. 16, 1920, from specimens from Iowa labeled *H. teres*.

H. No. 5. Isolated by F. L. Stevens Jan. 16, 1920, from barley, from specimen from Iowa labeled *H. gramineum*.

H. No. 6. From E. C. Stakman, Jan. 20, 1920. Isolated from blighted seedling of Marquis wheat.

H. No. 7. From E. C. Stakman, Jan. 20, 1920. Isolated from Marquis wheat.

H. No. 8. From E. C. S. (E. C. Stakman), Jan. 20, 1920. Isolated from Marquis wheat growing in sterile soil.

H. No. 9. From E. C. S., Jan. 20, 1920. Isolated from roots of Marquis wheat seedlings.

H. No. 10. From E. C. S., Jan. 20, 1920. Isolated from barley, Madison, Wis., "2-1919."

H. No. 11. From E. C. S., Jan. 20, 1920. Isolated in 1914 from wheat in Minnesota.

H. No. 12, labeled *H. gramineum*, from E. C. S., Jan. 20, 1920. Isolated from barley, Carver, Minn., 1914.

H. No. 13, labeled *H. sativum*. From L. W. Durrell, Feb. 6, 1920. From barley grown on the agronomy farm, Ames, Iowa. Isolated by Durrell about 1918 "from spots or lesions pointed out as typical by Dr. A. G. Johnson."

No. 14, labeled *H. sativum*. From L. W. Durrell, Feb. 6, 1920. From barley. Same origin as No. 13.

H. No. 15, labeled *H. teres*. From L. W. Durrell, Feb. 6, 1920, dated May 22, 1918. Same data as No. 13.

H. No. 16, labeled *H. teres*. Same data as for No. 13.

H. No. 17, labeled *H. gramineum*. Same data as for No. 13.

H. No. 18, labeled *H. avenae*. Same data as for No. 13, except that isolation was from oats, grown in rust nursery.

H. No. 19, labeled *H. gramineum*. From H. Coons, Feb. 16, 1920. Isolated from barley in 1918.

H. No. 20, labeled *H. teres*. From Centraal Bureau voor Schimmelcultures. Culture of Feb. 11, 1920. Isolated from late blight of barley by Bakke and sent in 1914 to Dr. Westerdijk, who wrote me that "the fungus had since been cultured on oatmeal (roll culture) and ears of barley."

H. No. 21, labeled *H. interseminatum*. From Centraal Bureau voor Schimmelcultures, March 12, 1920. Culture of Feb. 11, 1920. Received by Dr. Westerdijk from Miss Dale in 1912 and cultured on oatmeal or on corn-meal.

H. No. 22. From Wanda Weniger, Mar. 24, 1920. Isolated April 28, 1919, from kernels of Arnautka wheat in North Dakota. Used in field studies in 1919.

H. No. 23, labeled *H. teres*. From Wanda Weniger, Mar. 24, 1920. Isolated Feb. 27, 1920, from blade of barley collected at Mandan, N. Dak. July 1919.

H. No. 24. From Wanda Weniger, Mar. 24, 1920. Isolated Feb. 28, 1920, from first node of Red Durum wheat collected at Fargo, N. Dak., Aug. 1919.

H. No. 25. From Wanda Weniger, Mar. 24, 1920. Isolated Nov. 20, 1918, from blade of rye collected at Fargo, N. Dak. Used in field studies in 1919.

H. No. 26, labeled *H. sativum*. From Wanda Weniger, Mar. 24, 1920. Isolated July 12, 1919, from blade of wheat collected at Fargo.

H. No. 27. From Wanda Weniger, Mar. 24, 1920. Isolated from blade of "De" wheat collected at Fargo. Isolated Aug. 18, 1919, and used in field studies in 1919.

Cultures 22-27 were grown on 2 or 3% potato-agar with 2% dextrose from the time of their isolation until received by me. The species determinations were stated to be based on the host; not on morphological characters.

H. No. 29. From G. N. Hoffer. "Culture B. 201a." Isolated from a broken corn-stalk from Ft. Branch, Ind., Aug. 13, 1919.

H. No. 30. From G. N. H. (G. N. Hoffer). "Culture B. 180." Isolated from brown, water-soaked lesions on the sheath of a corn-leaf. Plant was collected at Sullivan, Ind., Aug. 12, 1919.

H. No. 31. From G. N. H. "Culture B. 170 A." Isolated from small brown spots on corn leaves. Collected at Delphi, Ind., Aug. 8, 1919.

H. No. 32. From G. N. H. "Culture B. 165." Was isolated from small yellow spots on corn leaves collected at Battle Ground, Ind., Aug. 7, 1919.

H. No. 34. From G. N. H. "Culture B. 124." Isolated from dark brown, irregularly shaped lesions on corn stalks collected at Ames, Iowa, July 25, 1919.

H. No. 36. From C. E. Kurtzweil. Labeled "3% oat 206 5-40." Isolated from a dead corn-stalk in Tennessee.

H. Nos. 37 and 38. Isolated by F. L. S. (F. L. Stevens) Aug. 8, 1920, from wheat grains after treatment with Javelle water.

H. No. 39. Isolated by F. L. S. Nov. 20, 1920, from Chaetochloa (millet). Conidium short, 3-septate.

H. No. 40. Isolated by F. L. S. Nov. 20, 1920, from same plant as No. 39. Conidium long, narrow.

H. No. 41. Isolated by F. L. S. Dec. 3, 1920, from sorghum.

H. No. 42. Isolated by F. L. S. Dec. 10, 1920, from millet.

H. No. 43. Isolated by F. L. S. Dec. 10, 1920, from sorghum.

H. No. 44. Isolated by W. L. Blain Dec. 20, 1920, from wheat.

H. No. 45. Isolated by F. L. S. from association with H. No. 44.

H. No. 46. From E. J. Butler, Mar. 10, 1921. Isolated from wheat by R. E. Massey in Khartoum in the Anglo-Egyptian Sudan. The statement is made that "the straw was completely rotted through the base, and broke off short when handled. The fungus was present in pure culture on the crown and roots."*

Discussion of foregoing List with several Brief Descriptions

H. Nos. 29-32, though perhaps not quite identical, agreed closely with each other. They were mostly 5-celled, with the central cell inequilateral and the two end-cells pale. In conidial measurements they approached rather closely to *H. inaequalis* Shear, *H. tritici* P. Henn., and *H. geniculatum* T. & E.

H. Nos. 13 and 14 were of identical parentage.

H. Nos. 15 and 16 were from one strain though separated by several transfers. The original strain (15) was sent because the growth (16) did not look characteristic.

H. No. 11 usually gave no conidia at all and was quite distinct in culture characters and in color and septation of conidia.

H. Nos. 36, 40, and 41 are closely alike in morphological characters. H. Nos. 40 and 41 differ from H. No. 36 in that they do not possess the abundant aerial mycelium. Nos. 40 and 41 differ in zonation and in amount of aerial mycelium, and all three of these forms differ somewhat in their conidial graphs. They also differ in mycelial characters and in the way in which they penetrate wheat cells, though all three do the latter vigorously, completely occupying the cells and causing rotting. The three had best be regarded as elementary species of the same Linneon.

Description of H. No. 36.—Conidia mostly long and slender (Pl. XXI,*a*) but very variable in length. Stipe short but distinct. Apex pale. No constrictions at the septa. Septa usually thick and obvious. Conidia tapering very slightly from point of maximum thickness toward each end; straight or slightly arched. Episporium brittle; endosporium gelatinous. Conidiophores uniform; sterile portion thin, slender, quite long (350 μ), about 4 μ thick, smooth, brown, cells 24-28 μ long, not constricted at the septa; fertile portion slightly thicker and darker, cells short, therefore geniculations crowded, growth on agar characterized by abundant aerial mycelium (Pl. X)—more abundant than in any other form studied—as was also evident under standard conditions.

*From letter from E. J. Butler dated Feb. 28, 1921.

Isolated from dead corn-stalk in Tennessee by C. E. Kurtzweil.

Data on conidial length of H. No. 36 are as follows:

Frequency,	1	2	3	3	6	11	12	10	12	17	21	9
Microns,	34	37.1	40.8	44.2	47.6	51	54.4	57.8	61.2	64.6	68	71.4
Frequency,	10	15	12	11	7	6	3	3	0	2	1	
Microns,	74.6	78.2	81.8	85	88.4	91.8	95.2	98.6	102	105.4	108.8	

Conidial breadth ranged uniformly from 10.2 to 13.6 microns.

Data on septation are as follows:

Frequency,	3	3	6	13	17	8	6	1	1
Septa,	4	5	6	7	8	9	10	11	12

Description of H. No. 39.—Conidia short, quite uniform in size, cylindrical or very slightly tapering from point of maximum thickness (Pl. XXI, *b*). Hilum usually evident. Setpa thick, usually three; no constriction. Apical spot barely perceptible. Episporium brittle. Endosporium gelatinous. Conidiophores uniform; sterile portion 190-250 μ long, smooth, 3.5 μ. thick, not constricted, cells about 17-24 μ long; fertile portion much darker and nearly twice as thick as the sterile part, genicula very congested, numerous, usually 35-70 μ long. Conidia remaining attached in very large clusters.

Data on conidial length are as follows:

Frequency,	1	1	1	2	13	19	19	9	3	2	0	1
Microns,	6.8	10.2	13.6	17	20.4	23.8	27.2	30.6	34	37.4	40.8	44.2

Conidial breadth was quite uniformly 10.2 μ.

Data on septation are as follows:

Frequency.....	3	1	31
Septa.....	1	2	3

This organism produced on wheat many infection points with the appressoria and "callus," but differed from H. No. 1 in the minute characters of the infection spot.

H. No. 46 is very closely like H. No. 39. Data on the conidial length of H. No. 46 are as follows:

Frequency.....	1	3	4	8	15	4	1
Microns.....	8	20.4	23.8	27.2	30.6	34	37.4

Conidial breadth was uniformly as follows:

Frequency.....	2	11	1
Microns.....	6.8	10.2	13.6

The data on septation are:

Frequency.....	1	15
Microns.....	2	3

General Explanation of Graphs

All graphs on a page are drawn to the same scale. In all graphs of length and breadth the class value is 3.4 μ. All computations are based on class values. These in case of length and breadth can be converted to microns by use of the factor 3.4, or by the following table of equivalents:

Class	Microns	Class	Microns	Class	Microns
1	3.4	13	44.2	25	85.
2	6.8	14	47.6	26	88.4
3	10.2	15	51.	27	91.8
4	13.6	16	54.4	28	95.2
5	17.	17	57.8	29	98.6
6	20.4	18	61.2	30	102.
7	23.8	19	64.6	31	105.4
8	27.2	20	68.	32	108.8
9	30.6	21	71.4	33	112.2
10	34.	22	74.8	34	115.6
11	37.4	23	78.2		
12	40.8	24	81.6		

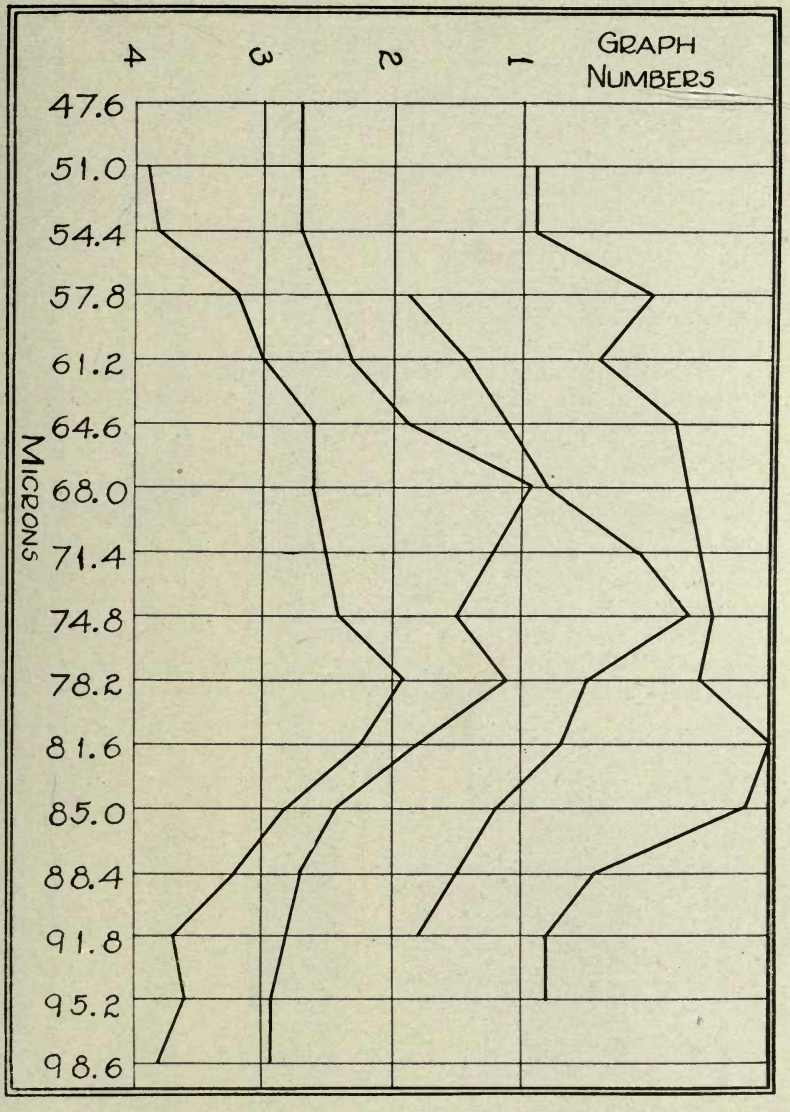
The customary symbols are used in presenting the data of the graphs, f, designating frequency; M, mean; σ, standard deviation; and CV, coefficient of variability.

FIGURE A

Conidial length of H. No. 1 grown on corn-meal agar made at different temperatures: Graph 1, on agar made at 100°; Graph 2, on agar made at 85°; Graph 3, on agar made at 60°; Graph 4, on agar made at 43°.

Graph	f	M	σ	CV
1	132	21.96 ± .16	2.84 ± .11	12.03 ± .54
2	113	21.96 ± .13	2.18 ± .09	9.93 ± .44
3	129	21.15 ± .17	2.89 ± .12	13.68 ± .58
4	149	22.02 ± .17	3.10 ± .12	14.09 ± .56

FIGURE A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78.2
81.6
85.0
88.4
91.8
95.2
98.6

FIGURE B

Conidial breadth of H. No. 1 grown on corn-meal agar made at different temperatures: Graph 5, on agar made at 60°; Graph 6, on agar made at 43°.

Graph	M	σ	CV
5	5.50 ± .04	.22 ± .03	4.06 ± .61
6	5.50 ± .03	.18 ± .02	3.31 ± .40

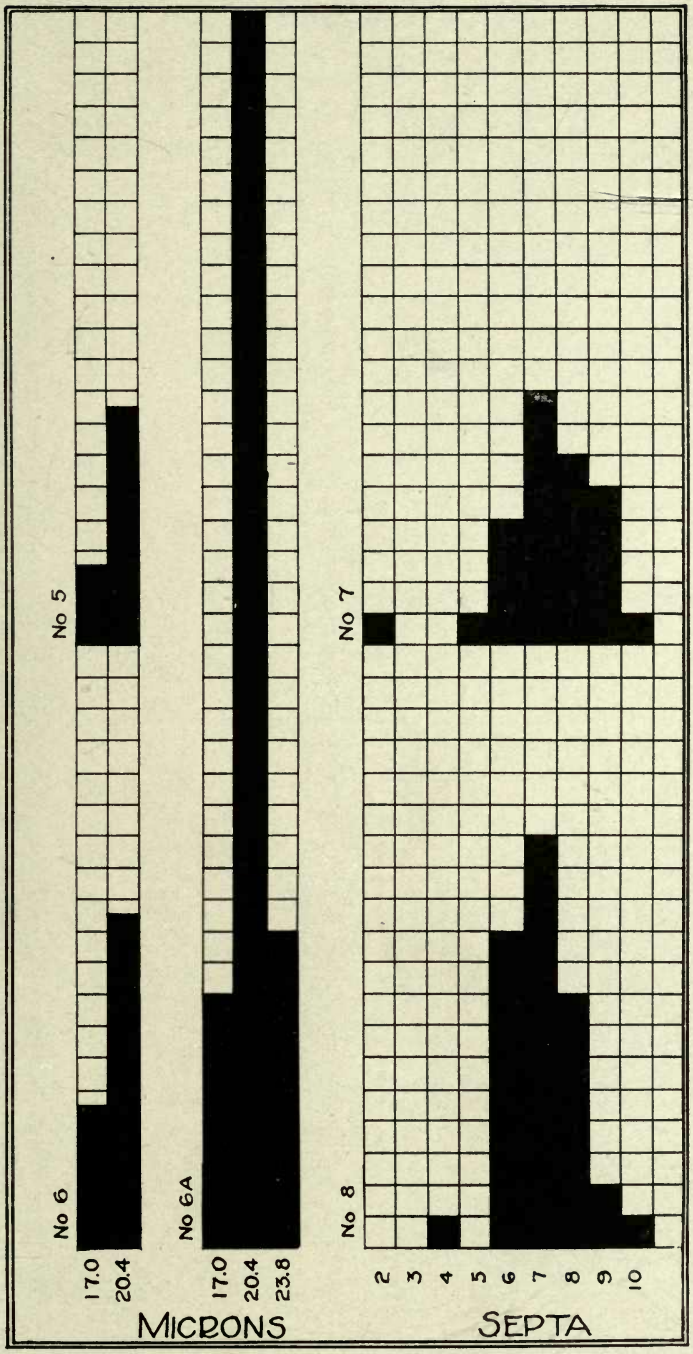
Graph 6A, conidial breadth of H. No.1 grown under standard conditions. (See app., p. 180).

Graph	f	M	σ	CV
6A	57	6.03 ± .04	0.55 ± .34	9.13 ± .57

Conidial septa of H. No. 1 grown on corn-meal agar: Graph 7, septa on agar made at 60°; Graph 8, septa on agar made at 43°.

Graph	M	σ	CV
7	7.30 ± .21	1.58 ± .14	21.72 ± 2.12
8	7.05 ± .12	1.11 ± .09	15.86 ± 1.31

FIGURE B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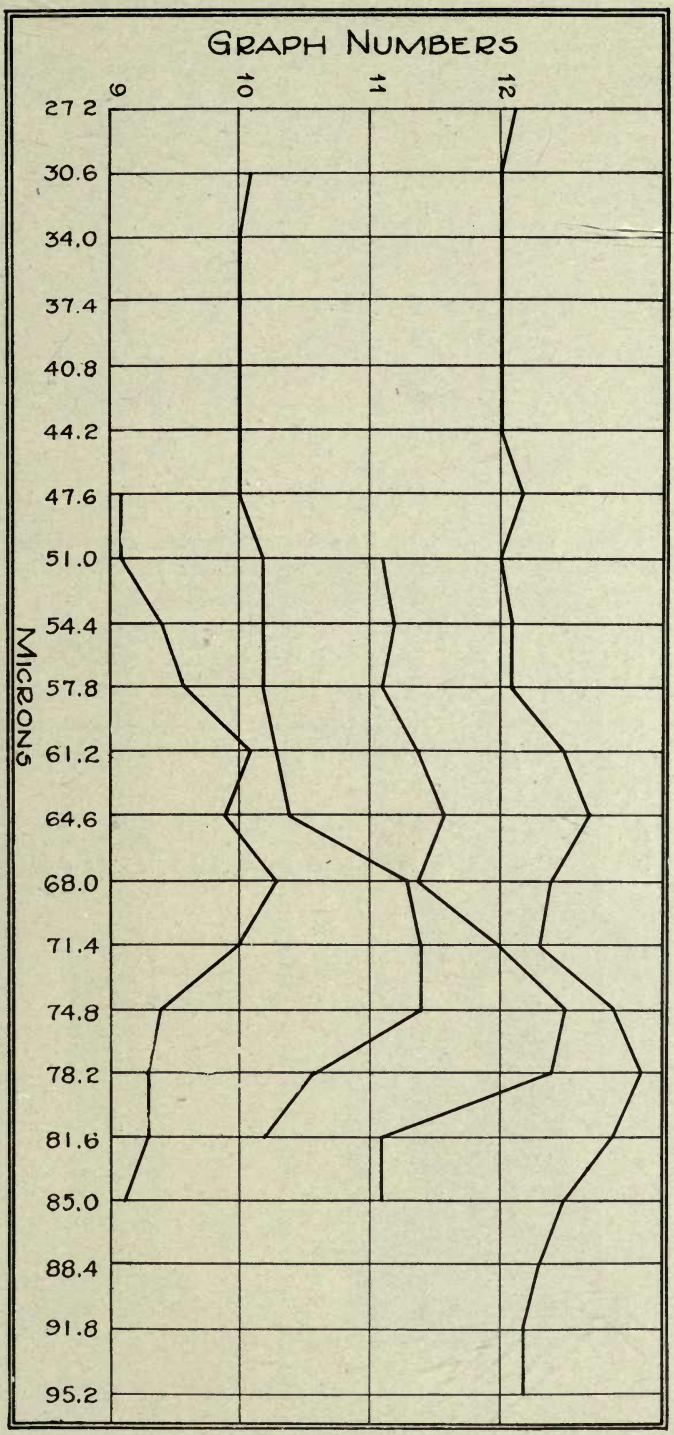
No 6
No 6A
No 7
No 8
17.0
20.4
17.0
20.4
23.8
MICRONS
2
3
4
5
6
7
8
9
10
SEPTA

FIGURE C

Conidial length of H. No. 1 when grown on plain agar and on agar with various amounts of corn-meal agar: Graph 9, length on plain agar; Graph 10, on plain agar ¾, plus ¼ corn-meal agar; Graph 11, on plain agar ½, plus ½ corn-meal agar; Graph 12, on plain agar ¼ plus ¾ corn-meal agar.

Graph	f	M	σ	CV
9	66	19.51 ± .18	2.27 ± .13	11.67 ± .61
10	63	20.36 ± .21	2.47 ± .14	12.13 ± .73
11	59	21.03 ± .18	2.10 ± .13	10.01 ± .62
12	65	21.76 ± .29	3.51 ± .20	16.15 ± .98

FIGURE C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64.6
68.0
71.4
74.8
78.2
81.6
85.0
88.4
91.8
95.2
MICRONS

FIGURE D

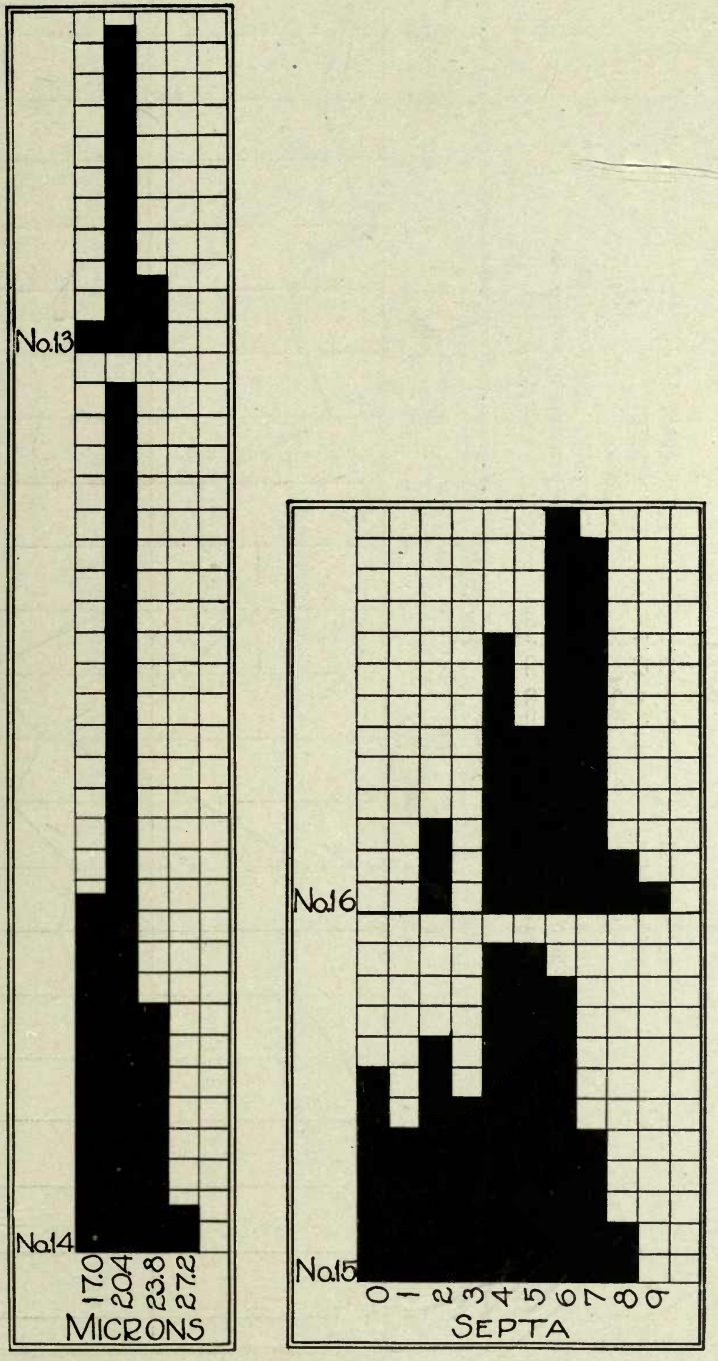
Conidial breadth of H. No. 1 grown on green-wheat agar of different compositions: Graph 13, on washed agar ¼, green-wheat agar ¾; Graph 14, on washed agar ¾, green-wheat agar ¼.

Graph	f	M	σ	CV
13	14	6.10 ± .06	0.38 ± .04	6.32 ± .80
14	44	5.98 ± .07	0.71 ± .05	11.87 ± .86

Conidial septa of H. No. 1: Graph 15, grown on washed agar ¼, green-wheat agar ¾; Graph 16, grown on washed agar ¾, green-wheat agar ¼.

Graph	f	M	σ	CV
15	65	3.83 ± .18	2.22 ± .13	58.02 ± 4.10
16	46	5.63 ± .15	1.56 ± .11	27.80 ± 2.10

FIGURE D


No.13
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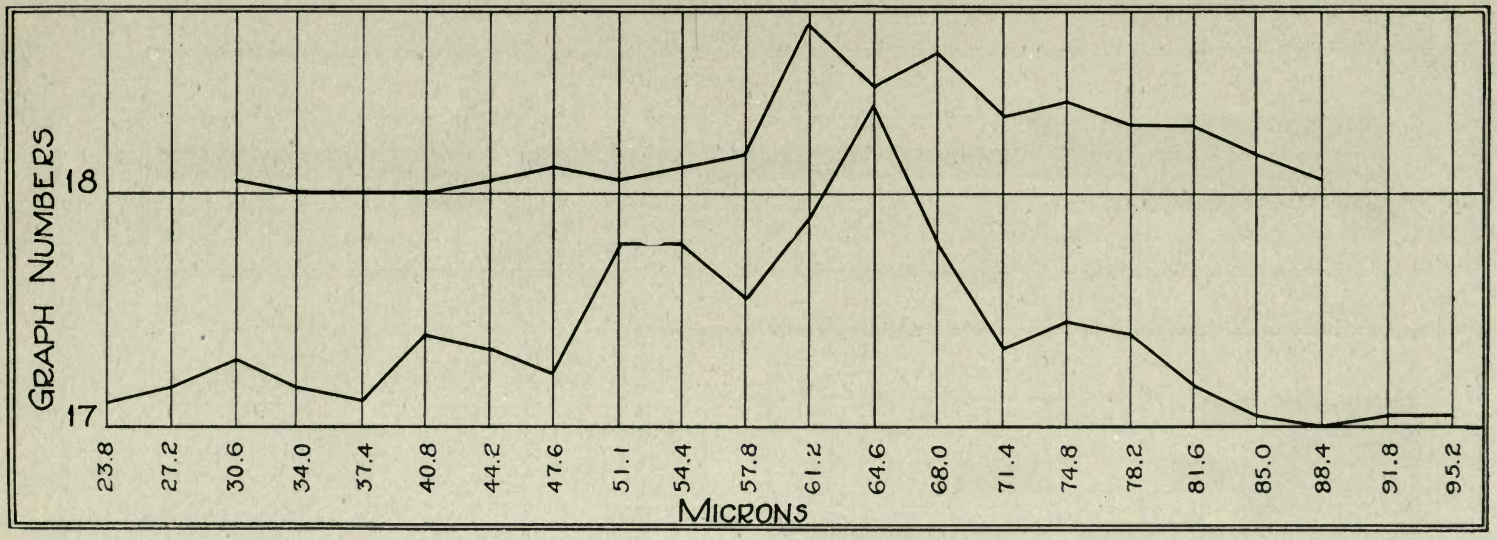
27.2
MICRONS
No.16
No.15
0 1 2 3 4 5 6 7 8 9
SEPTA

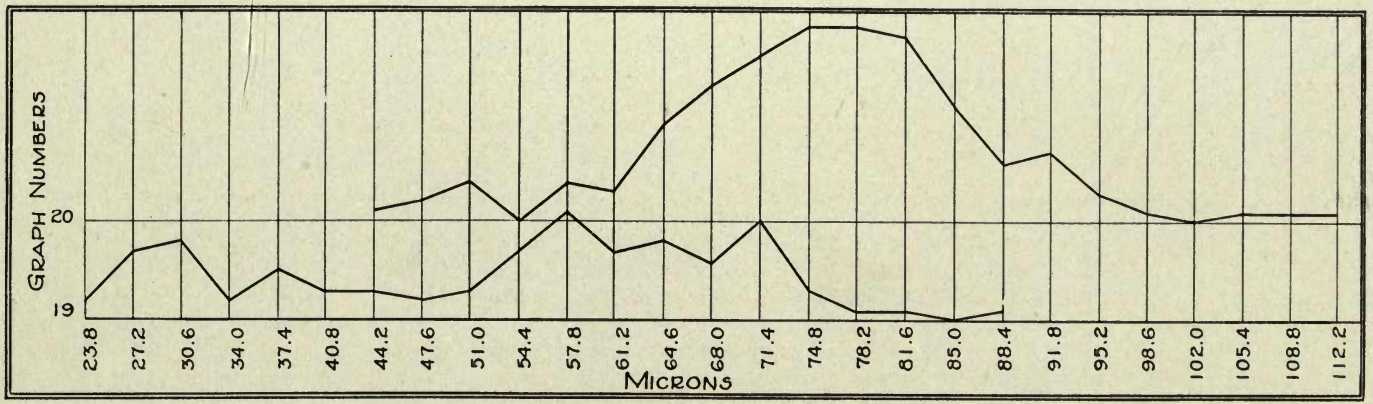
FIGURE E



Conidial length of H. No. 1 grown on corn-meal agars of different quantities in standard 100 mm.-Petri-dishes: Graph 17, on 66 c.c. of agar; Graph 18, on 12 c.c. of agar.

Graph	M	σ	CV
17	17.20 ± .22	4.03 ± .15	23.45 ± .95
18	19.85 ± .25	3.07 ± .17	15.51 ± .91

FIGURE F



Conidial length of H. No. 1 on old wheat straw at different humidities: Graph 19, grown in comparatively dry conditions near the top of the test-tube; Graph 20, grown in humid conditions near the bottom of the tube.

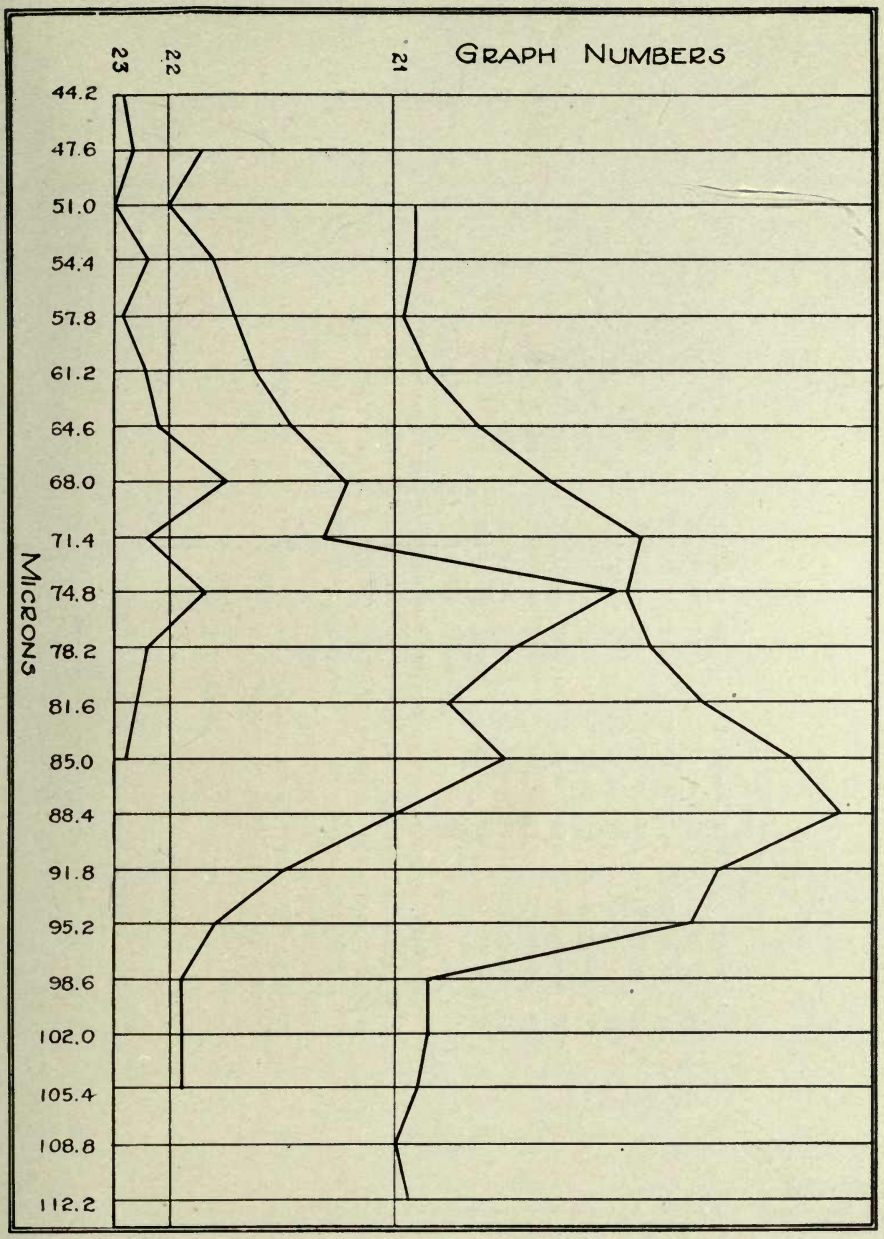
Graph	f	M	σ	CV
19	——	15.67 ± .33	4.77 ± .24	30.46 ± 1.66
20	147	22.39 ± .18	3.04 ± .13	15.22 ± .61

FIGURE G

Conidial length of H. No. 1 grown on live wheat shoots at three temperatures: Graph 21, fungus grown at 15°; Graph 22, fungus grown at 20°; Graph 23, fungus grown at 30°.

Graph	f	M	σ	CV
21	265	24.30 ± .12	3.01 ± .08	12.38 ± .36
22	225	22.71 ± .13	2.97 ± .09	13.08 ± .42
23	41	19.92 ± .29	2.76 ± .20	13.86 ± 1.05

FIGURE G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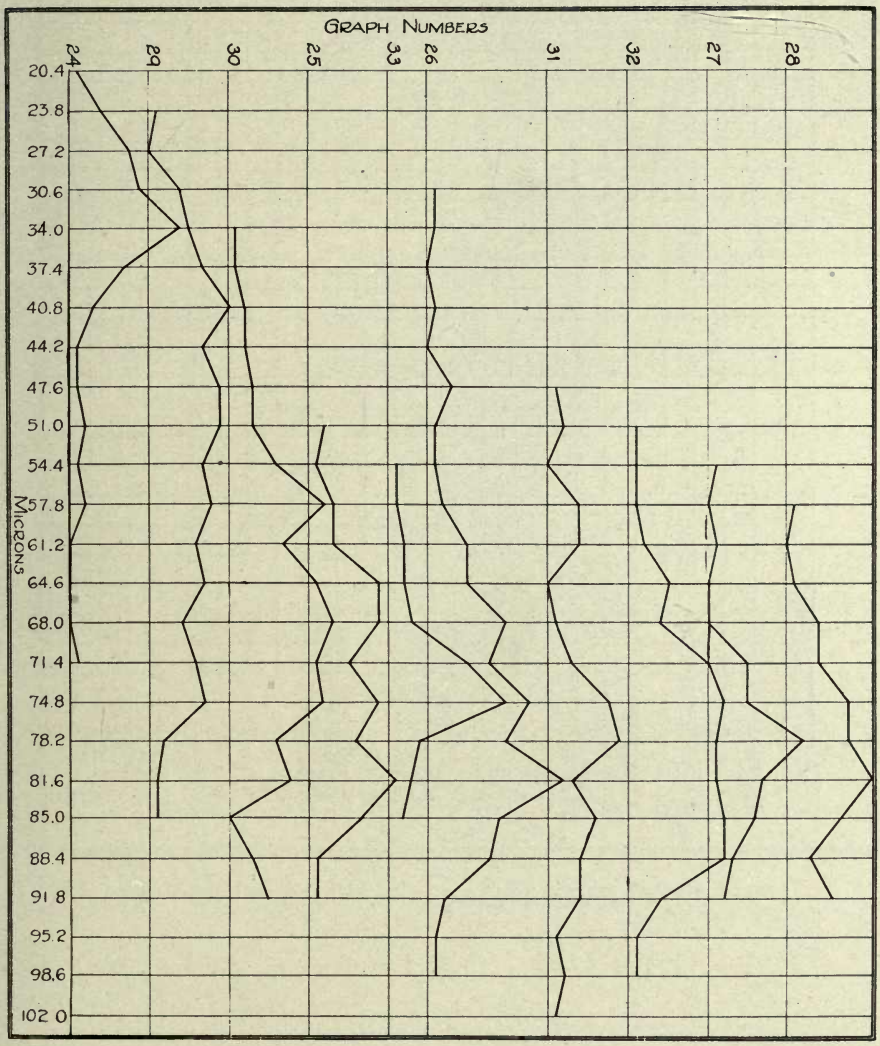
78.2
81.6
85.0
88.4
91.8
95.2
98.6
102.0
105.4
108.8
112.2

Figure H

Conidial length of H. No. 1 grown on plain agar with various nutrients added: Graph 24, with saccharose added; Graph 25, with "buckwheat flour" added; Graph 26, with corn meal added; Graph 27, with wheat flour added; Graph 28, with corn-starch added; Graph 29, tapioca added; Graph 30, rice added; Graph 31, Brazil-nut fragments added; Graph 32, wheat fragments added. Graph 33, conidial length in the region of inhibition near the edge of the colony on plain agar.

Graph	f	M	σ	CV
24	54	10.37 ± .26	2.83 ± .18	27.35 ± 1.19
25	67	21.41 ± .21	2.64 ± .15	12.37 ± .15
26	99	21.80 ± .24	3.66 ± .17	16.81 ± .82
27	42	23.21 ± .21	2.11 ± .15	9.09 ± .66
28	53	33.37 ± .20	2.24 ± .14	9.59 ± .62
29	101	15.70 ± .27	4.09 ± .19	26.08 ± 1.31
30	106	19.72 ± .23	3.65 ± .16	18.48 ± .88
31	53	22.64 ± .34	3.75 ± .24	16.59 ± 1.11
32	88	23.03 ± .19	2.73 ± .13	11.87 ± .61
33	43	21.41 ± .19	1.88 ± .13	8.79 ± .63

FIGURE H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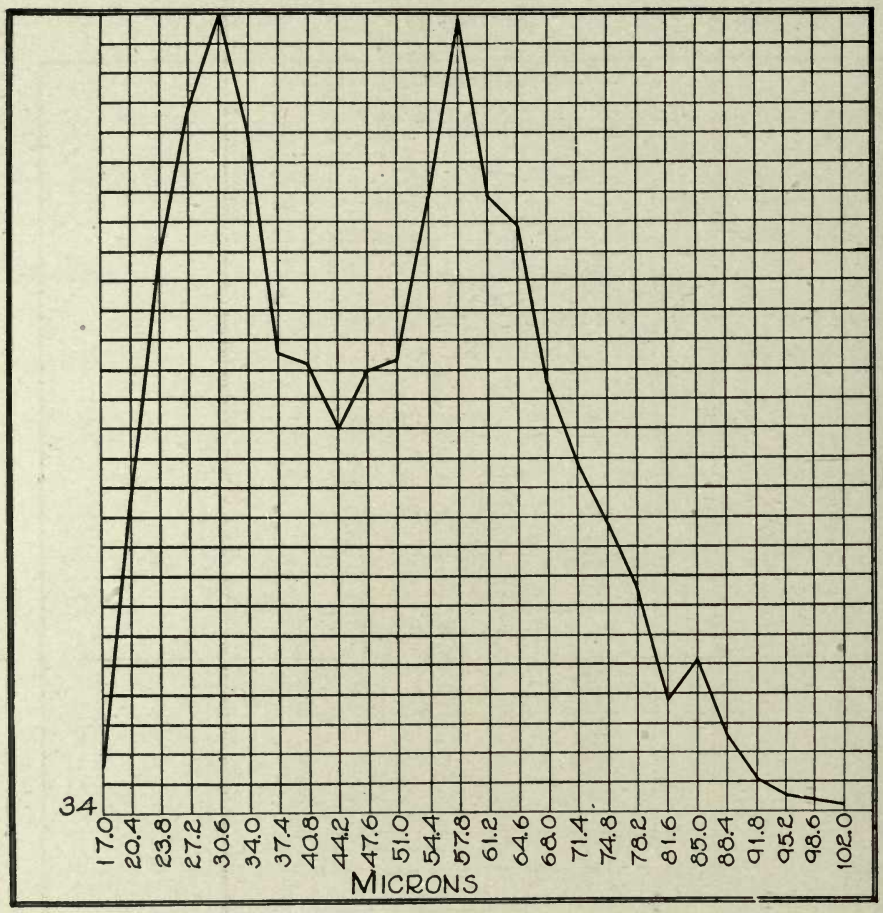
98.6
102.0

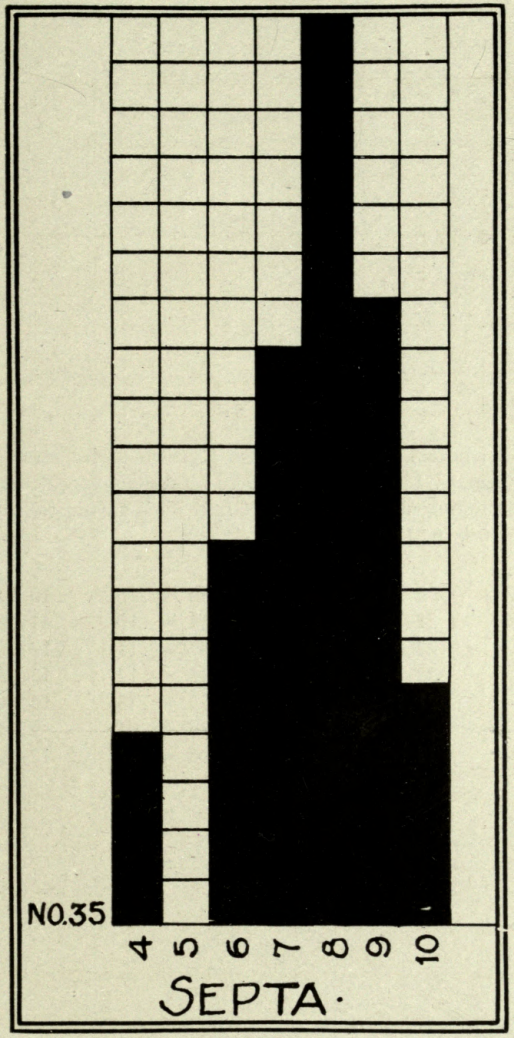
FIGURE I



Length of 1646 conidia of H. No. 1 grown on corn-meal agar. See also pp. 120-121.

Graph	M	σ	CV
34	14.34 ± .08	5.35 ± .06	37.35 ± .70

FIGURE J



Conidial septation of H. No. 1.

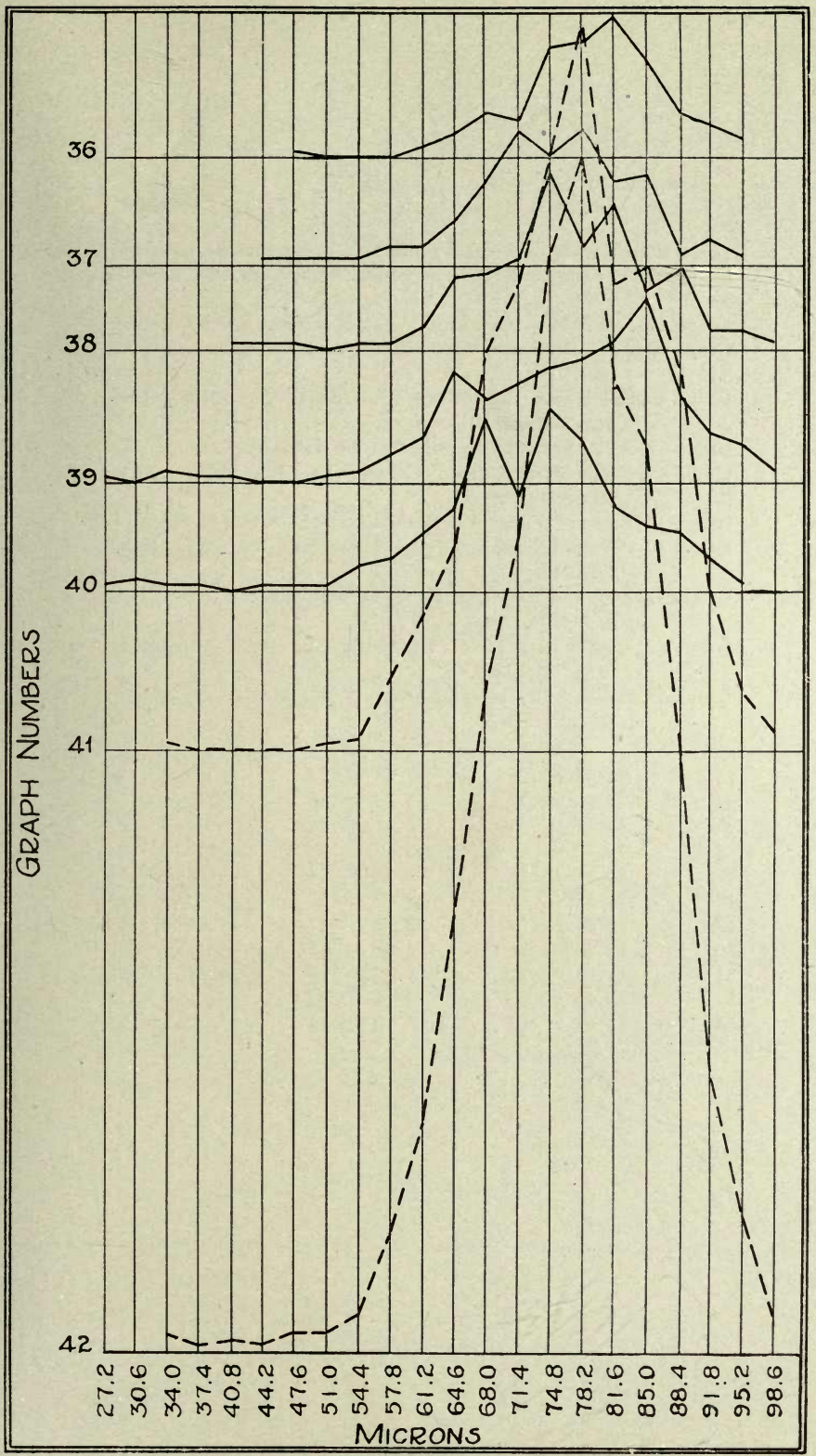
Graph	f	M	σ	CV
35	58	7.91 ± .08	.99 ± .—	12.51 ± .79

FIGURE K

Graphs of conidial length of H. No. 1 grown under standard conditions (see app. p. 180): Graphs 36-40 represent respectively plates a-e; Graph 41 represents plate e′; and Graph 42 is a composite of plates a, b, c, d, e′ (see p. 120).

Graph	f	M	σ	CV
36	123	23.21 ± .15	2.36 ± .10	10.02 ± .46
37	107	22.51 ± .15	2.54 ± .10	11.28 ± .49
38	142	22.59 ± .16	2.87 ± .11	12.70 ± .51
39	180	22.42 ± .17	3.42 ± .12	15.25 ± .55
40	—	21.18 ± .18	3.55 ± .13	16.80 ± .63
41	647	22.71 ± .05	2.26 ± .04	9.95 ± .01
42	1199	22.62 ± .05	2.76 ± .03	12.22 ± .16

FIGURE K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61.2
64.6
68.0
71.4
74.8
78.2
81.6
85.0
88.4
91.8
95.2
98.6
MICRONS

FIGURE L

Graphs of conidial length of H. No. 2 (H. ravenelii).

Graph 43, Seymour and Earle, Economic Fungi, No. 399, Florida, 1890.

Graph 44, Ellis, North Americam Fungi, No. 368. North Carolina.

Graph 45, A. B. Seymour's specimen as grown by me on corn-meal agar.

Graph 46, deThümen, Mycotheca Universalia, No. 1468. Carolina, 1876.

Graph 47, A. B. Seymour's specimen from Louisiana, 1919.

Graph 48, Bartholomew, Fungi Columbiana, No. 3026. Nova Scotia, 1909.

Graph 49, Ravenel, Fungi Americani Exsiccati, No. 165. Florida.

Graph 50, Ellis and Everhart, Fungi Columbiani, No. 4633. Florida, 1914.

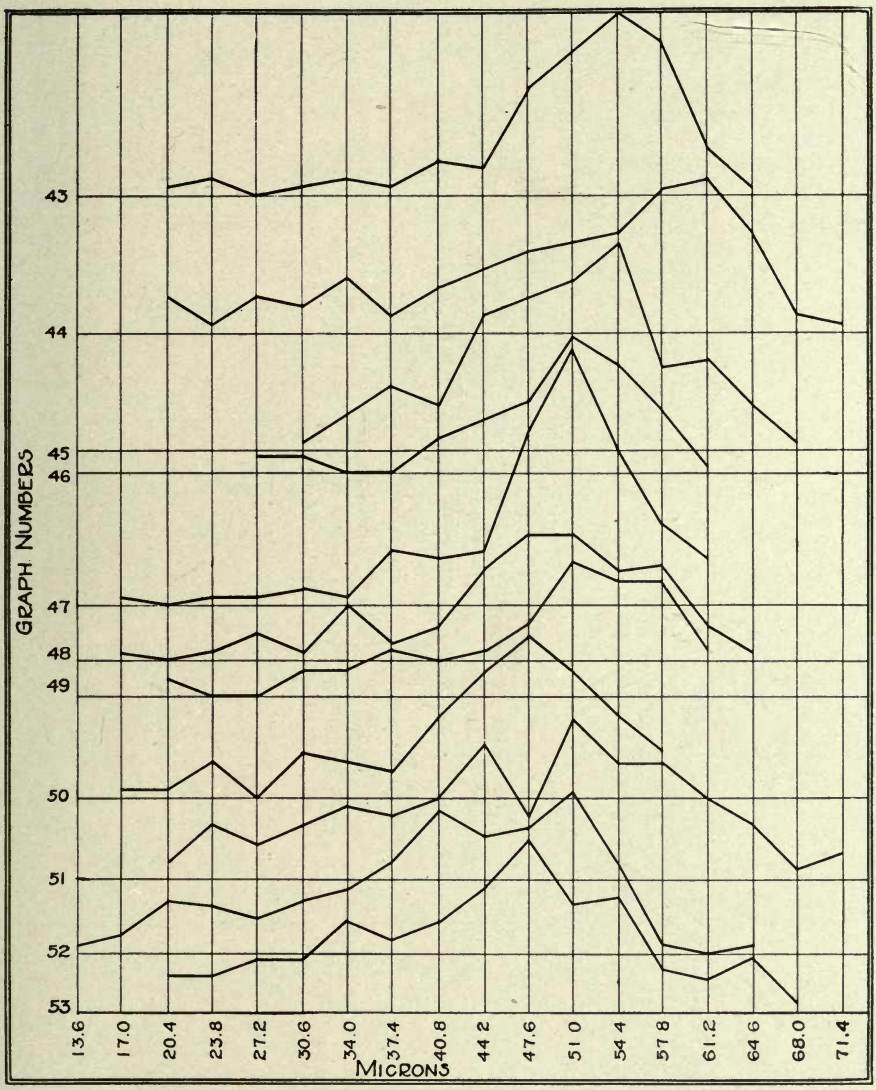
Graph 51, Ellis and Everhart, Fungi Columbiani, No. 465. Carolina, 1894.

Graph 52, Rabenhorst, Fungi Europeai, No. 3082. Argentine, 1878, sample 1.

Graph 53, Rabenhorst, Fungi Europeai, No. 3082a. Argentine, 1878, sample 2.

Graph	M	σ	CV
43	14.97 ± .18	2.53 ± .13	16.89 ± .89
44	14.80 ± .22	3.58 ± .15	24.17 ± 1.73
45	14.79 ± .14	2.27 ± .10	15.34 ± .69
46	14.49 ± .19	2.19 ± .16	15.11 ± .97
47	14.38 ± .16	2.38 ± .11	16.54 ± .84
48	14.35 ± .22	3.02 ± .15	21.04 ± 1.15
49	14.34 ± .21	2.74 ± .15	19.10 ± 1.18
50	13.98 ± .20	2.82 ± .14	20.15 ± 1.50
51	13.78 ± .20	3.49 ± .14	25.31 ± 1.08
52	13.02 ± .20	3.16 ± .14	24.25 ± 1.18
53	12.05 ± .19	3.10 ± .13	25.72 ± 1.22

FIGURE L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40.8
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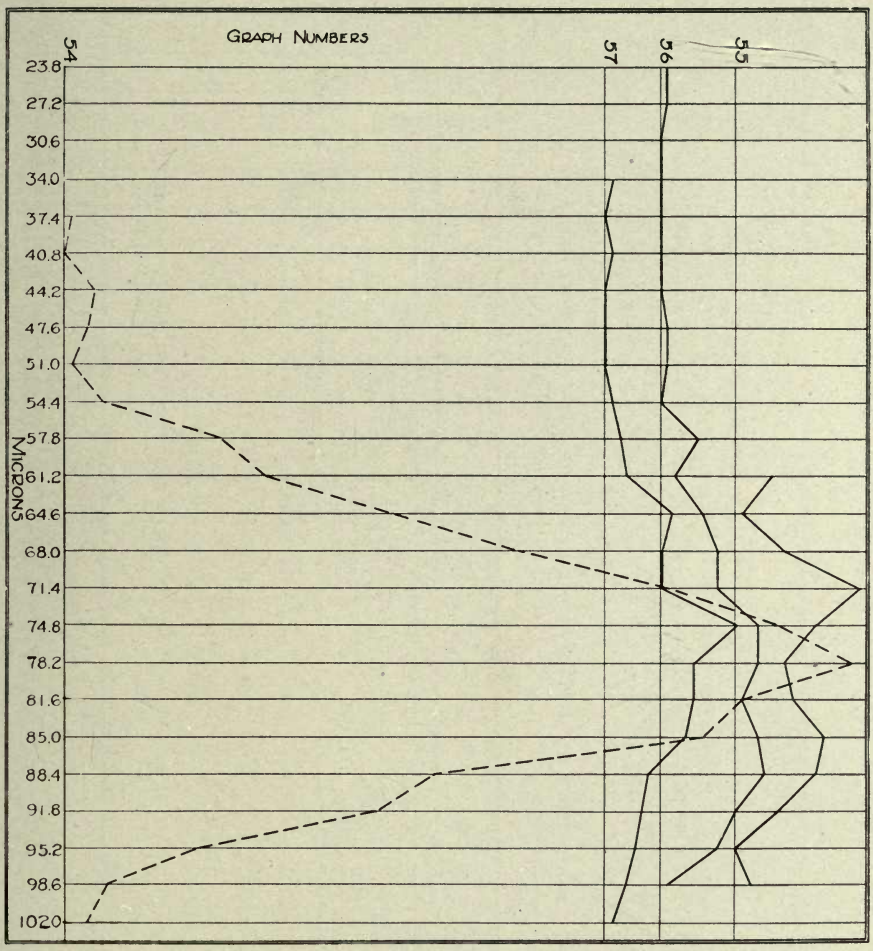
47.6
51.0
54.4
57.8
61.2
64.6
68.0
71.4
MICRONS

FIGURE M

Conidial length of H. No. 1 grown under standard conditions but on various cereal shoots: Graph 54, as grown on corn; Graph 55, as grown on rye; Graph 56, as grown on barley; Graph 57, as grown on wheat.

Graph	M	σ	CV
54	22.49 ± .06	2.83 ± .04	12.57 ± .20
55	23.06 ± .19	2.64 ± .13	11.44 ± .59
56	23.00 ± .19	3.09 ± .13	13.43 ± .60
57	22.66 ± .22	3.36 ± .15	14.82 ± .69

FIGURE M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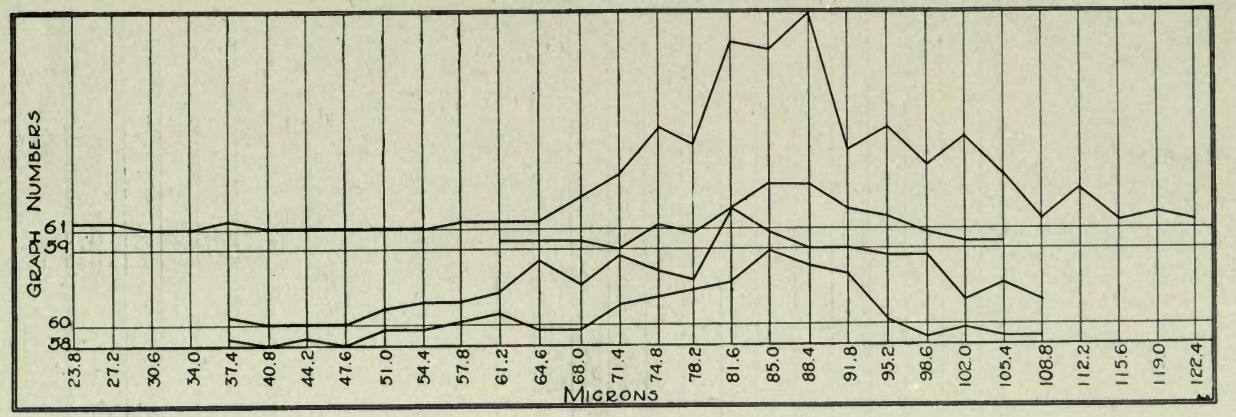
MICRONS

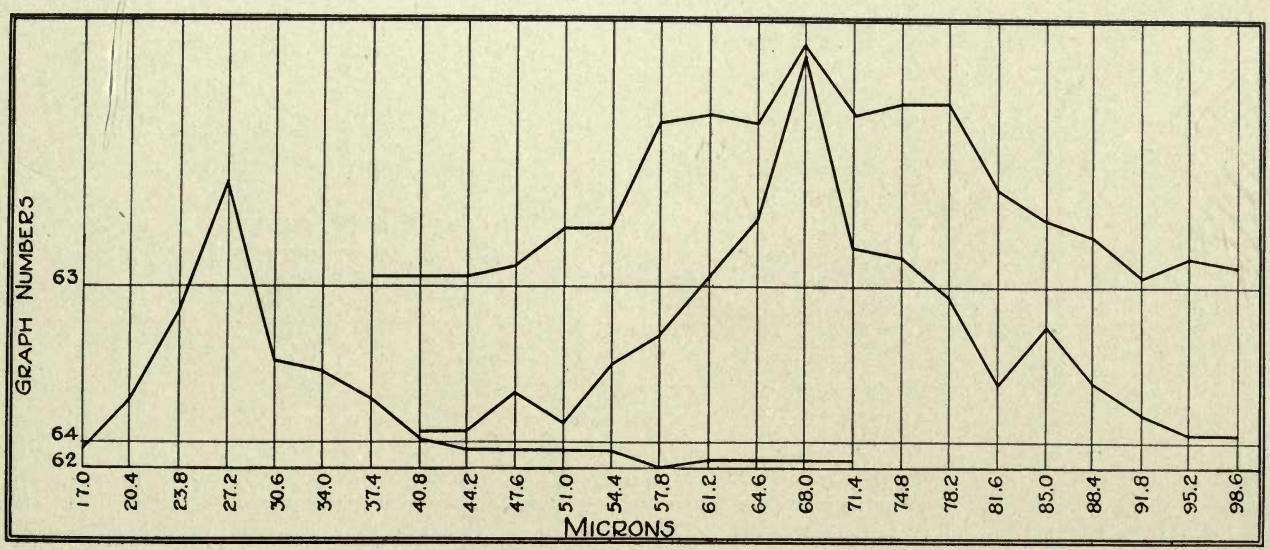
FIGURE N



Conidial length of H. No. 1: Graph 58, length on fresh wheat-stems; Graph 59, length on wheat leaves; Graph 60, on wheat shoots; Graph 61, on wheat plants.

Graph	f	M	σ	CV
58	82	23.36 ± .30	4.10 ± .21	17.58 ± .95
59	42	25.30 ± .27	2.68 ± .19	10.60 ± .78
60	124	24.10 ± .25	4.27 ± .18	17.72 ± .78
61	175	25.64 ± .20	4.04 ± .14	15.79 ± .58

FIGURE O



Three graphs (62, 63, 64) of conidial length of H. No. 1: Graph 62, length of conidia in bank of same produced near edge of colony grown on corn-meal agar in Petri dish, but dried till growth had stopped; Graph 63, length on washed agar ¾, plus green-wheat agar ¼; Graph 64, on live wheat from rag doll.

Graph	f	M	σ	CV
62	95	8.95 ± .18	2.68 ± .13	29.99 ± 1.59
63	—	20.35 ± .16	3.29 ± .11	16.19 ± .59
64	—	20.30 ± .14	2.97 ± .10	14.62 ± .51

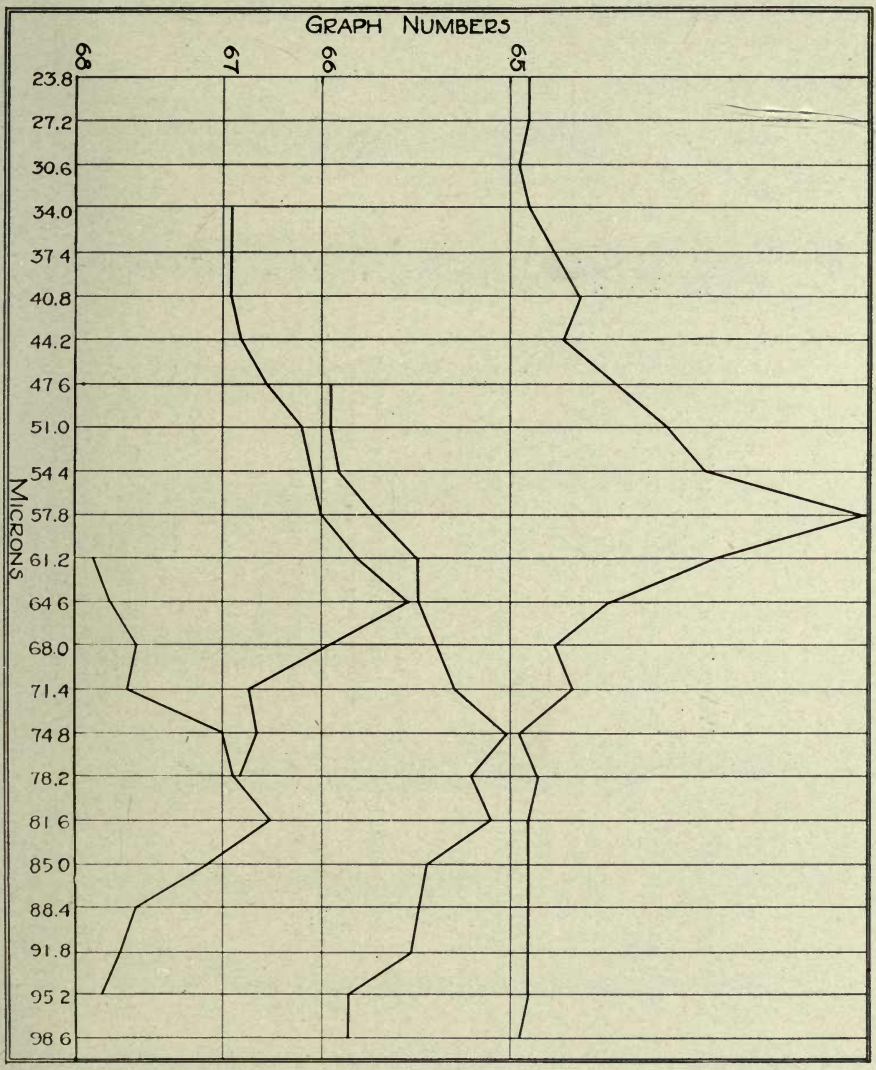
FIGURE P

Conidial length of M35, M36, and M40, with that of H. No. 1 for comparison: Graph 65, M35-1; Graph 66, M36-2; Graph 67, M40-2; Graph 68, H. No. 1.

Graph	f	M	σ	CV
65	179	16.80 ± .18	3.75 ± .13	22.35 ± .83
66	156	22.31 ± .16	3.14 ± .12	14.09 ± .54
67	97	17.70 ± .17	2.51 ± .12	14.19 ± .70
68	123	23.21 ± .15	2.36 ± .10	10.02 ± .46
	*1199	22.62 ± .05	2.76 ± .03	12.22 ± .16

*H. No. 1 of Graph 42 (Fig. K). See also p. 120.

FIGURE P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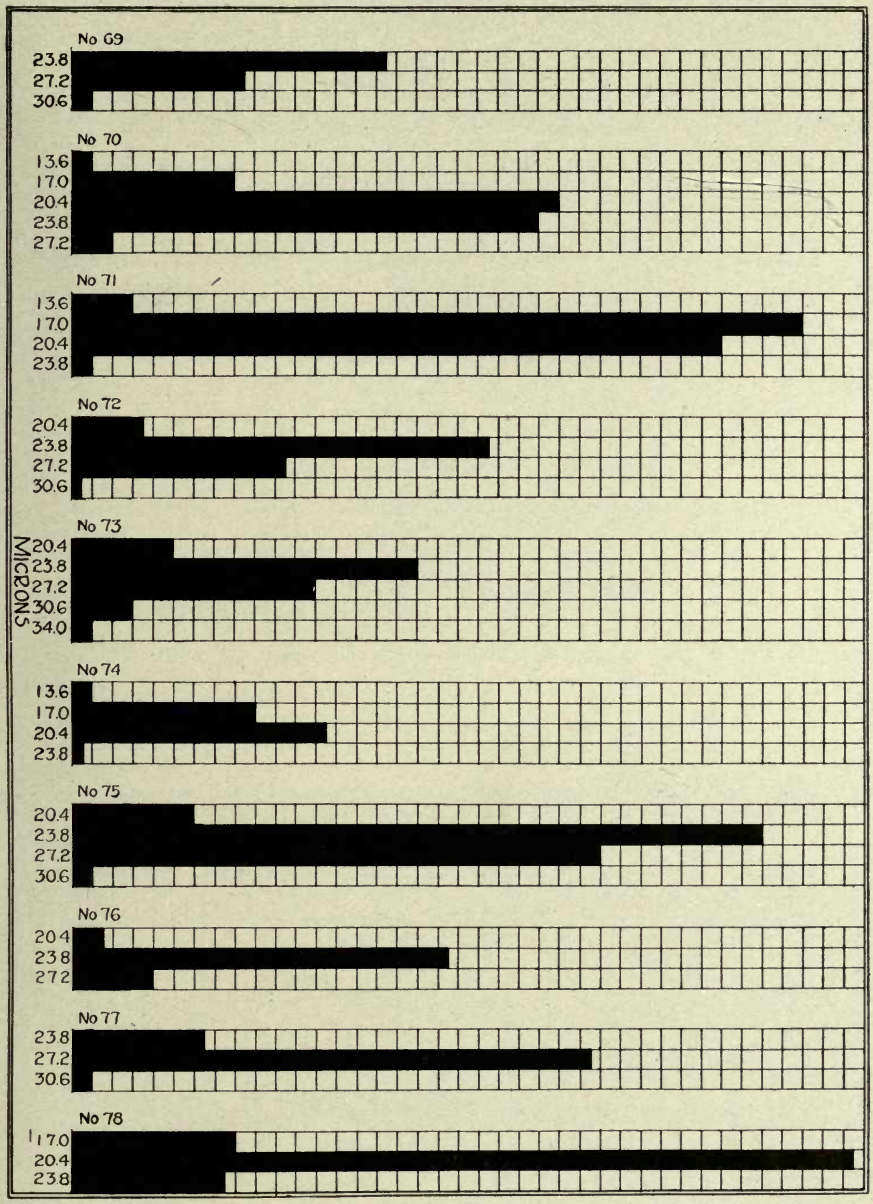
78.2
81.6
85.0
88.4
91.8
95.2
98.6

FIGURE Q

Graphs of conidial breadth of saltants and originals under standard conditions: Graph 69, M1-5; Graph 70, M6-1; Graph 71, original from same plate as M6-1; Graph 72, M6-5, a week later; Graph 73, M8-3; Graph 74, original from same plate as M8-3; Graph 75, M8-7; Graph 76, M8-10; Graph 77, M36-2; Graph 78, H. No. 1.

Graph	f	M	σ	CV
69	25	7.50 ± .07	.52 ± .05	7.05 ± .67
70	58	6.29 ± .07	.80 ± .05	12.86 ± .81
71	72	5.43 ± .04	.59 ± .03	10.99 ± .62
72	35	7.22 ± .06	.56 ± .04	7.81 ± .63
73	37	7.45 ± .10	.91 ± .07	12.30 ± .97
74	23	5.54 ± .07	.50 ± .05	9.18 ± .91
75	67	7.32 ± .04	.58 ± .03	8.04 ± .46
76	24	7.10 ± .06	.45 ± .04	6.41 ± .62
77	33	7.83 ± .03	.31 ± .02	4.05 ± .33
78	57	6.03 ± .04	.55 ± .34	9.13 ± .57

FIGURE Q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No 77
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27.2
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No 78
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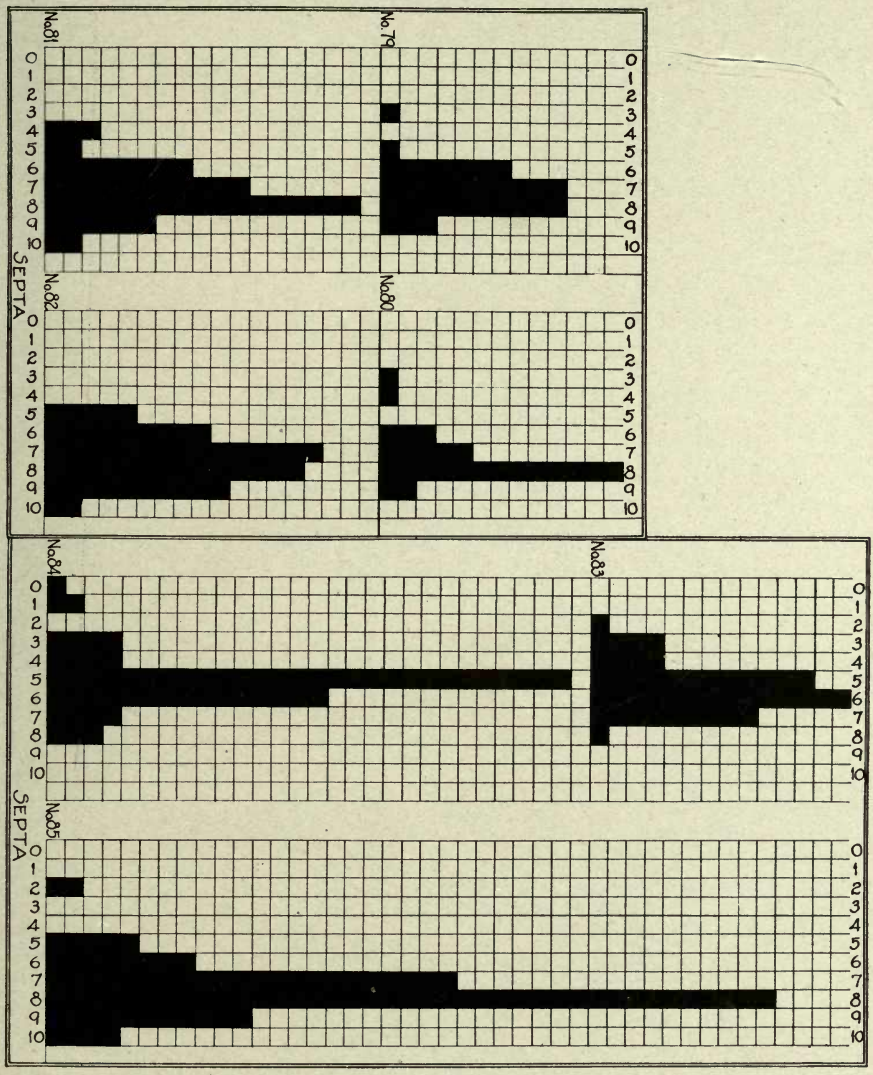
20.4
23.8

FIGURE R

Graphs of conidial septation of saltants: Graph 79, M4-6; Graph 80, M5-5; Graph 81, M6-5; Graph 82, M37-2; Graph 83, M1-5; Graph 84, M12-3; Graph 85 .M12-4.

Graph	f	M	σ	CV
79	32	7.09 ± .14	1.23 ± .10	17.39 ± 1.51
80	25	7.28 ± .18	1.37 ± .13	18.84 ± 1.86
81	49	7.28 ± .13	1.41 ± .09	19.41 ± 1.37
82	55	7.38 ± .11	1.30 ± .08	17.61 ± 1.16
83	45	5.44 ± .13	1.32 ± .09	24.35 ± 1.83
84	61	5.11 ± .12	1.48 ± .09	28.99 ± 1.91
85	81	7.43 ± .10	1.45 ± .07	19.57 ± 1.07

FIGURE R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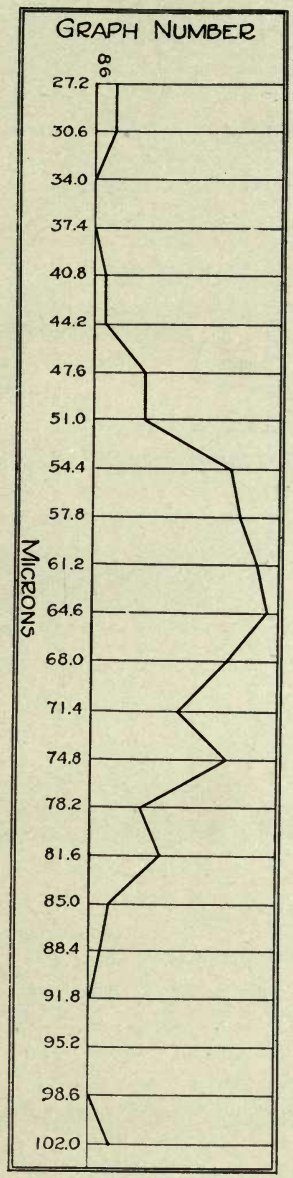
SEPTA
0 1 2 3 4 5 6 7 8 9 10

FIGURE S

Graph of conidial length of H. No. 11.

Graph	f	M	σ	CV
86	134	18.85 ± .20	3.58 ± .14	19.03 ± .81

FIGURE S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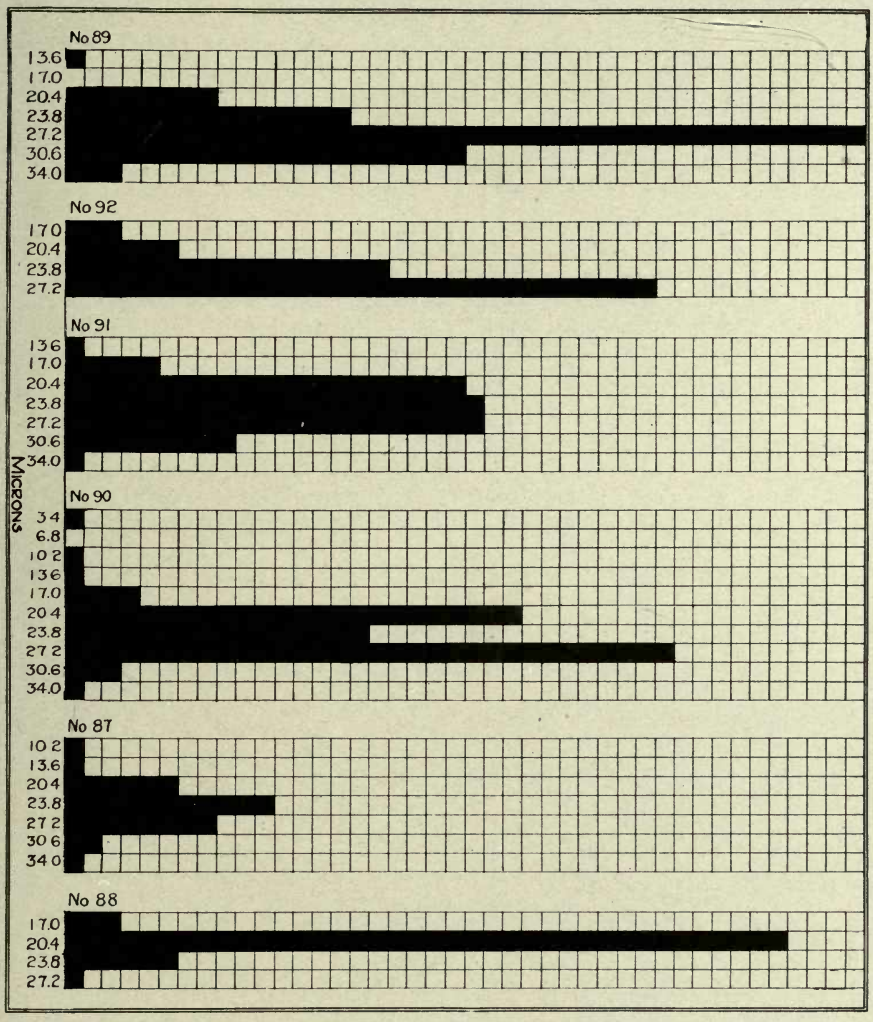
64.6
68.0
71.4
74.8
78.2
81.6
85.0
88.4
91.8
95.2
98.6
102.0

FIGURE T

Graphs of conidial septation of several Helminthosporiums under standard conditions: Graph 87, H. No. 11; Graph 88, H. No. 20; Graph 89, H. No. 13; Graph 90, H. No. 14; Graph 91, H. No. 15; Graph 92, H. No. 16.

Graph	f	M	σ	CV
87	30	6.13 ± .14	1.20 ± .10	19.62 ± 1.77
88	48	6.10 ± .04	.50 ± .03	8.35 ± .57
89	90	7.90 ± .07	1.02 ± .05	13.01 ± .66
90	83	6.95 ± .10	1.36 ± .07	19.58 ± 1.06
91	81	7.11 ± .08	1.19 ± .16	16.82 ± .91
92	57	7.33 ± .07	.86 ± .05	11.79 ± .75

FIGURE T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34.0
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No 91
13.6
17.0
20.4
23.8
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34.0
MICRONS
No 90
3.4
6.8
10.2
13.6
17.0
20.4
23.8
27.2
30.6
34.0
No 87
10.2
13.6
20.4
23.8
27.2
30.6
34.0
No 88
17.0
20.4
23.8
27.2

FIGURE U

Graphs of conidial length of several Helminthosporiums under standard conditions: Graph 93, H. No. 14; Graph 94, H. No. 13; Graph 95, H. No. 15; Graph 96, H. No. 16; Graph 97, H. No. 17; Graph 98, H. No. 18; Graph 99, H. No. 19; Graph 100, H. No. 20.

Graph	f	M	σ	CV
93	404	22.04 ± .10	3.03 ± .07	13.76 ± .33
94	597	24.78 ± .09	3.53 ± .06	14.24 ± .28
95	461	22.54 ± .10	3.49 ± .07	15.51 ± .35
96	445	23.75 ± .12	3.80 ± .08	16.03 ± .37
97	252	24.39 ± .15	3.63 ± .10	14.88 ± .45
98	97	23.03 ± .28	4.10 ± .19	17.81 ± .88
99	205	24.59 ± .19	4.15 ± .13	16.89 ± .57
100	315	18.84 ± .12	3.30 ± .08	17.53 ± .48

FIGURE U

98
99
97
94
95
96
93
100
GRAPH NUMBERS
6.8
10.2
13.6
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20.4
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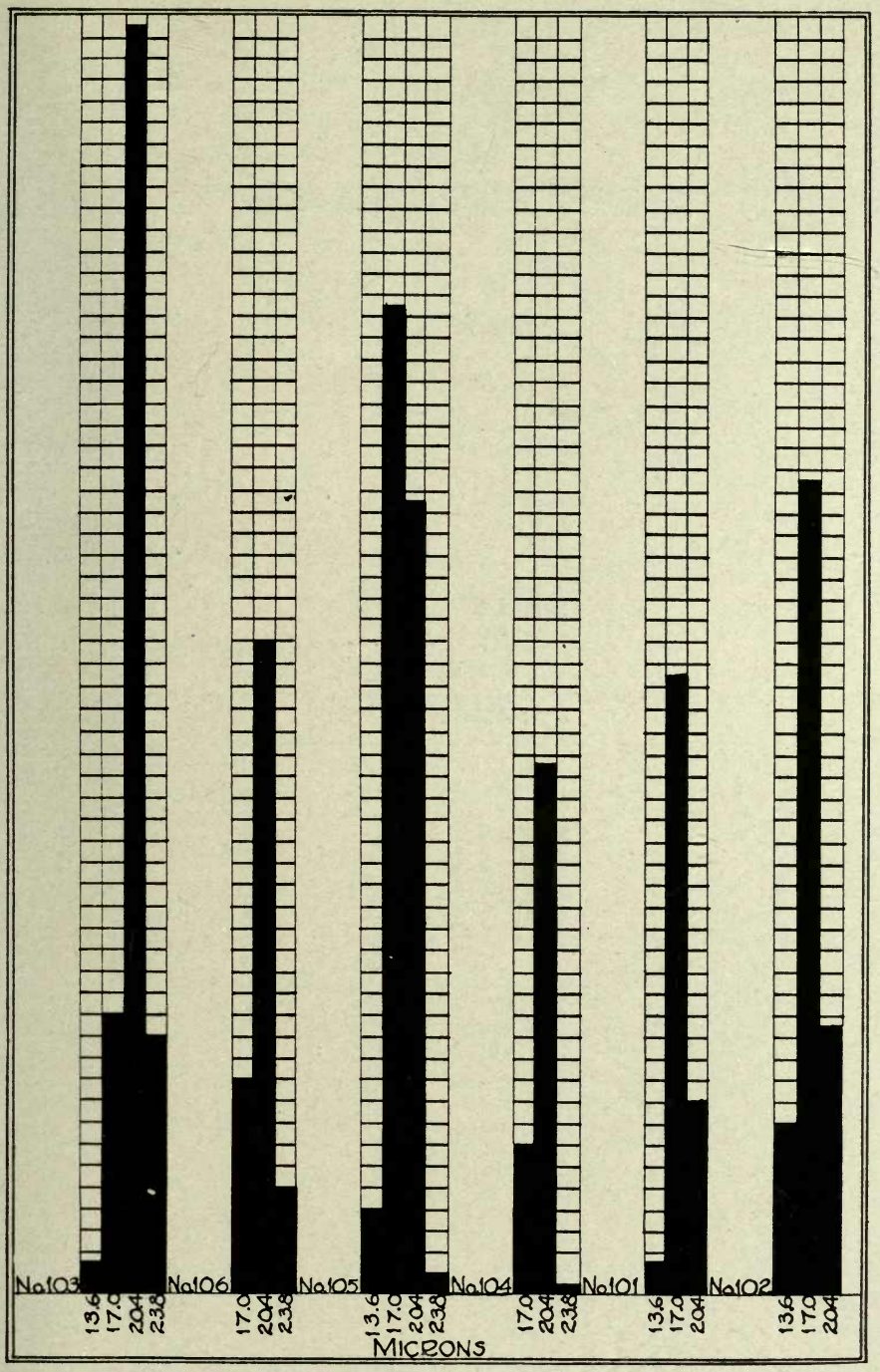
81.6
85.0
88.4
91.8
95.2
98.6
102.0
105.4
108.8
112.2
115.6
119.0
MICRONS

FIGURE V

Graphs of conidial breadth of several Helminthosporiums under standard conditions: Graph 101, H. No. 11; Graph 102, H. No. 20; Graph 103, H. No. 13; Graph 104, H. No. 14; Graph 105, H. No. 15; Graph 106, H. No. 16.

Graph	f	M	σ	CV
101	39	5.19 ± .04	.40 ± .03	7.74 ± .59
102	58	5.11 ± .14	.52 ± .03	10.27 ± .65
103	79	5.97 ± .08	1.06 ± .05	17.85 ± .98
104	32	5.59 ± .08	.74 ± .06	13.30 ± 1.14
105	88	5.39 ± .04	.56 ± .02	10.50 ± .53
106	45	5.88 ± .05	.56 ± .04	9.61 ± .68

FIGURE V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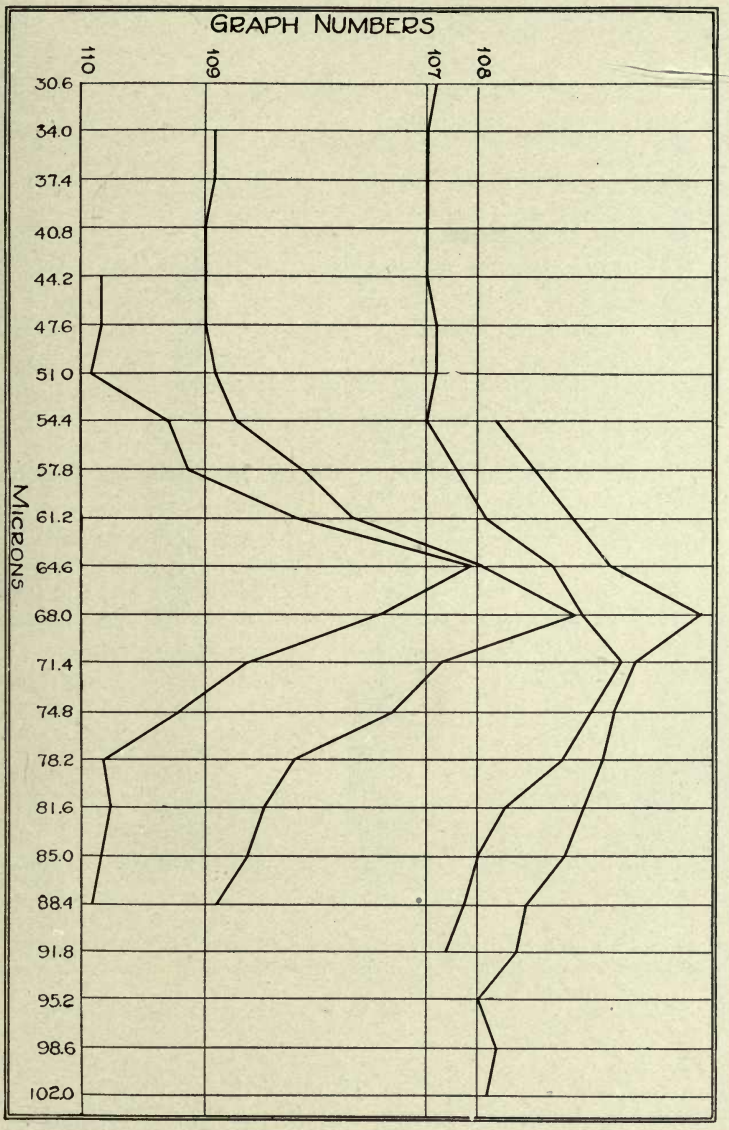
No104
17.0
20.4
23.8
No101
13.6
17.0
20.4
No102
13.6
17.0
20.4
MICRONS

FIGURE W

Graphs of conidial length—under standard conditions—of H. No. 1*a* (107); H. No. 1*b* (108); H. No. 1*c* (109); H. No. 1*d* (110).

Graph	f	M	σ	CV
107	111	21.20 ± .17	2.66 ± .12	12.58 ± .57
108	130	21.52 ± .16	2.86 ± .11	13.30 ± .56
109	160	20.09 ± .12	2.29 ± .08	11.42 ± 43
110	153	19.26 ± .11	2.12 ± .08	11.03 ± .43

FIGURE W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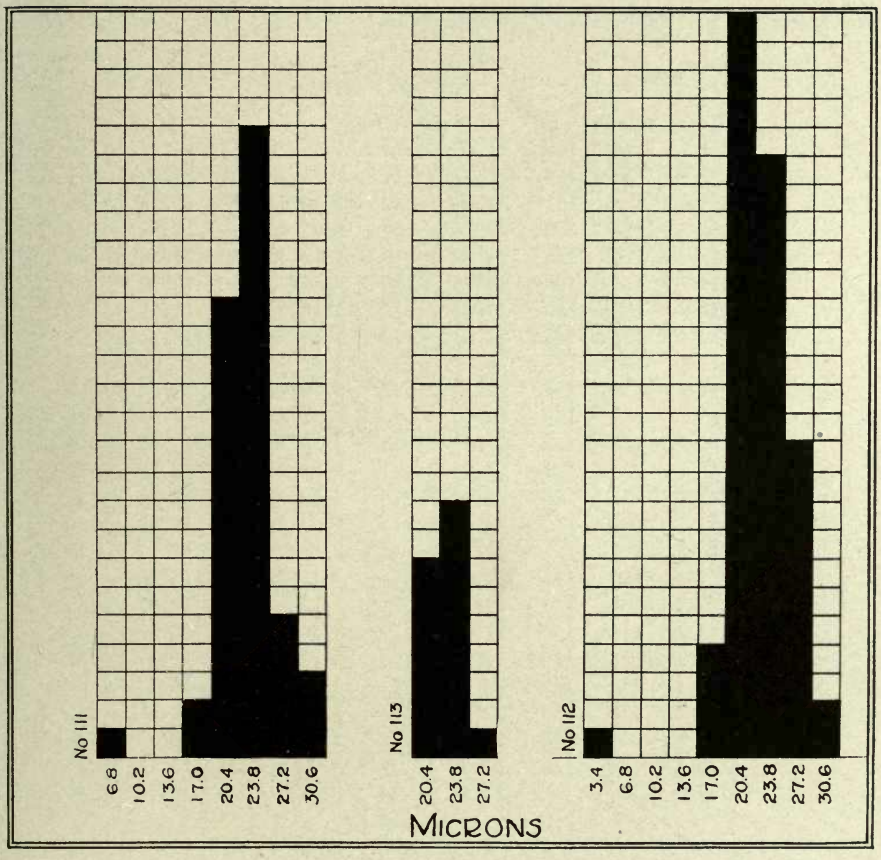
74.8
78.2
81.6
85.0
88.4
91.8
95.2
98.6
102.0
MICRONS

FIGURE X

Graphs of conidial septation—under standard conditions—of H. No. 1*a* (111); H. No. 1*b* (112); H. No. 1*c* (113).

Graph	f	M	σ	CV
111	49	6.71 ± .10	1.12 ± .07	16.75 ± 1.17
112	65	6.61 ± .09	1.15 ± .06	17.52 ± 1.08
113	17	6.64 ± .09	.58 ± .06	8.85 ± 1.02

FIGURE X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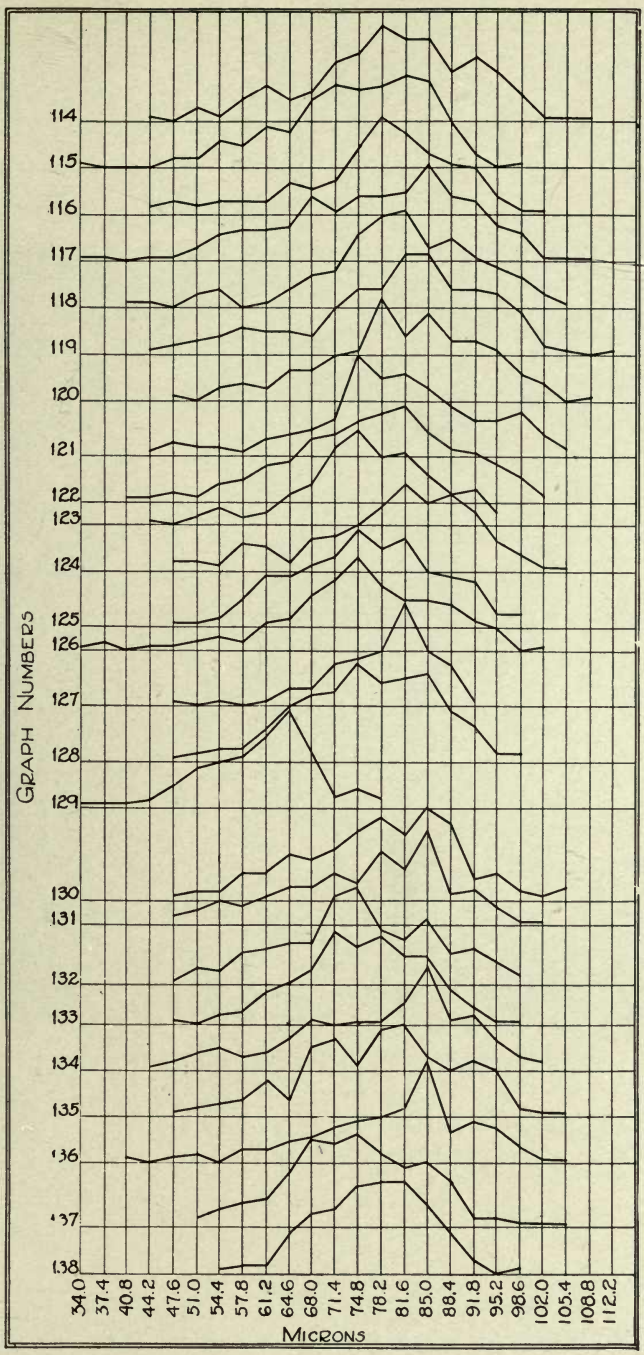
30.6
No 113
20.4
23.8
27.2
No 112
3.4
6.8
10.2
13.6
17.0
20.4
23.8
27.2
30.6
MICRONS

FIGURE Y

Saltant	Graph	f	M	σ	CV
M1-5	114	160	23.40 ± .18	3.53 ± .13	15.11 ± .58
M2-4	115	154	21.77 ± .17	3.17 ± .12	14.56 ± .57
M3-4	116	131	22.66 ± .20	3.50 ± .14	15.48 ± .66
M4-6	117	168	22.72 ± .21	4.04 ± .14	17.80 ± .67
M5-5	118	145	23.72 ± .19	3.52 ± .13	14.86 ± .60
M6-5	119	166	23.65 ± .20	3.85 ± .14	16.28 ± .61
M8-8	120	156	23.92 ± .18	3.38 ± .12	14.35 ± .55
M12-3	121	148	23.64 ± .20	3.73 ± .14	15.81 ± .63
M13-3	122	173	22.73 ± .18	3.61 ± .13	15.90 ± .69
M14-3	123	126	22.32 ± .17	2.98 ± .12	13.37 ± .57
M15-3	124	134	23.51 ± .20	3.51 ± .14	14.93 ± .62
M36-2	125	156	22.31 ± .16	3.14 ± .12	14.09 ± .54
M37-2	126	131	22.03 ± .21	3.64 ± .15	16.55 ± .70
M32-2	127	86	23.00 ± .16	2.33 ± .12	10.14 ± .52
M33-2	128	155	22.37 ± .16	3.02 ± .11	13.52 ± .52
M40-2	129	97	17.70 ± .17	2.51 ± .12	14.19 ± .70
M41-2	130	148	22.92 ± .19	3.45 ± .13	15.05 ± .60
M42-2	131	125	22.36 ± .21	3.53 ± .15	15.81 ± .69
M43-2	132	139	22.00 ± .19	3.36 ± .13	15.29 ± .63
M44-2	133	134	22.18 ± .15	2.72 ± .11	12.27 ± .51
M45-2	134	142	22.88 ± .21	3.76 ± .15	16.43 ± .67
M46-2	135	158	22.84 ± .17	3.34 ± .12	14.66 ± .56
M47-2	136	112	23.53 ± .22	3.56 ± .16	15.12 ± .69
M48-2	137	146	22.00 ± .16	3.02 ± .11	13.78 ± .55
M17-3	138	128	22.56 ± .14	2.38 ± .10	10.56 ± .45

Figure Y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74.8
78.2
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88.4
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95.2
98.6
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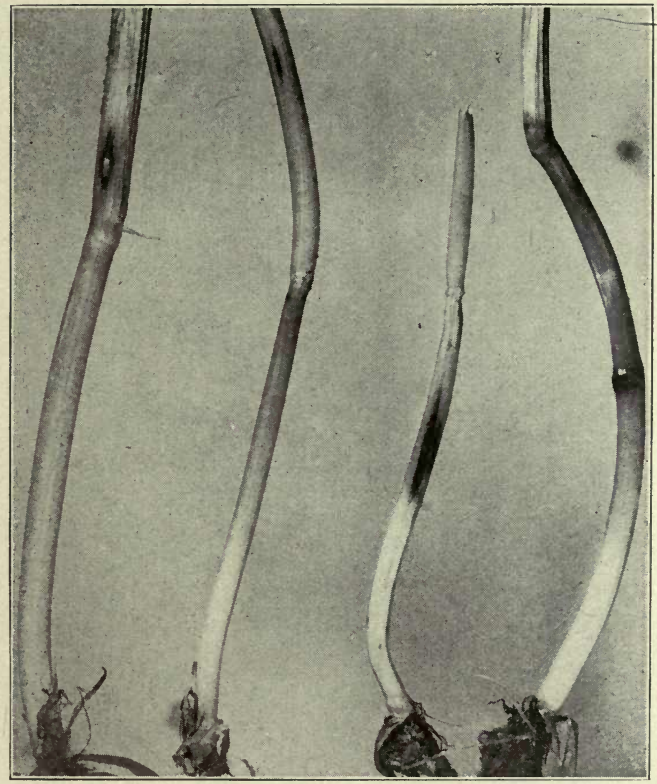
105.4
108.8
112.2
Microns

Plate VII

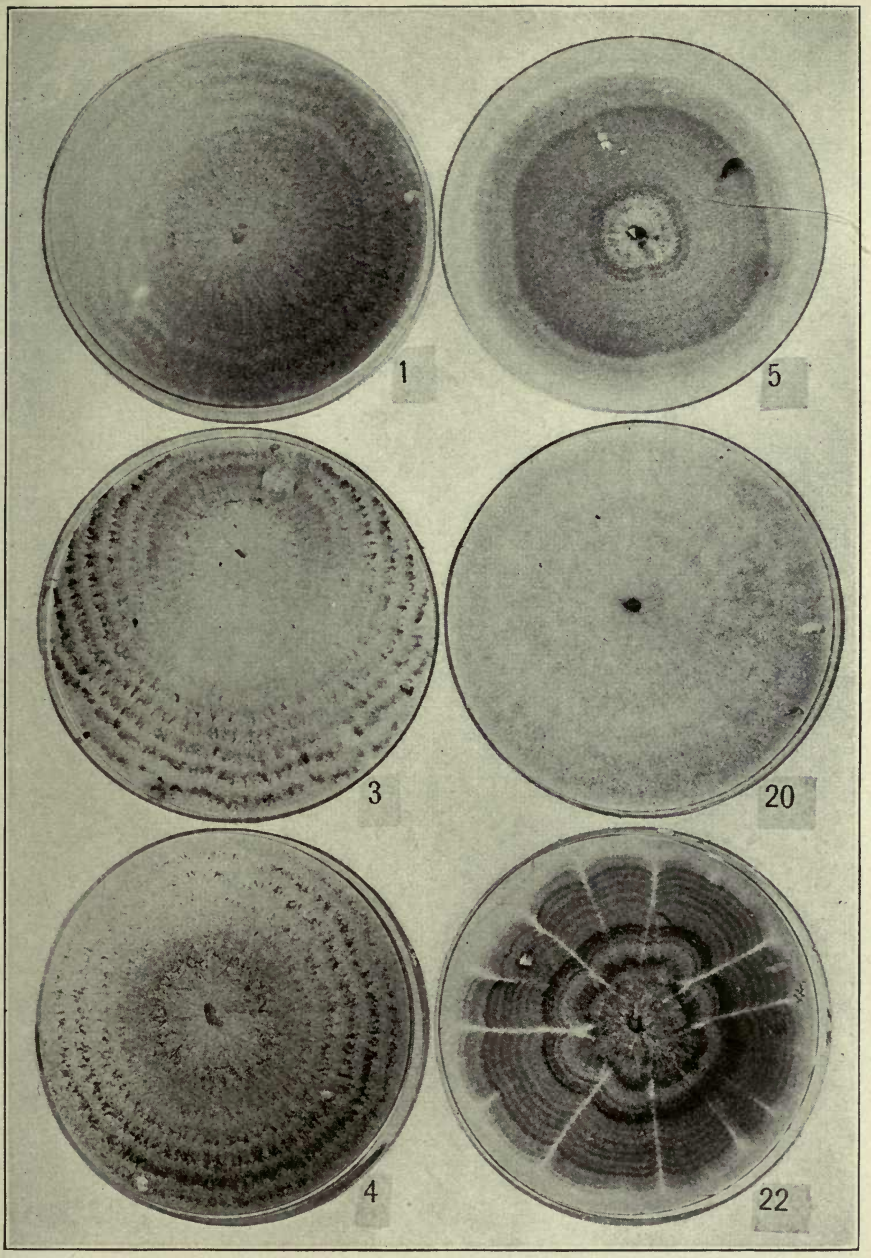


Several wheat stems showing characteristic diseased spots; also diseased portion at the node in one shoot.

Plate VIII

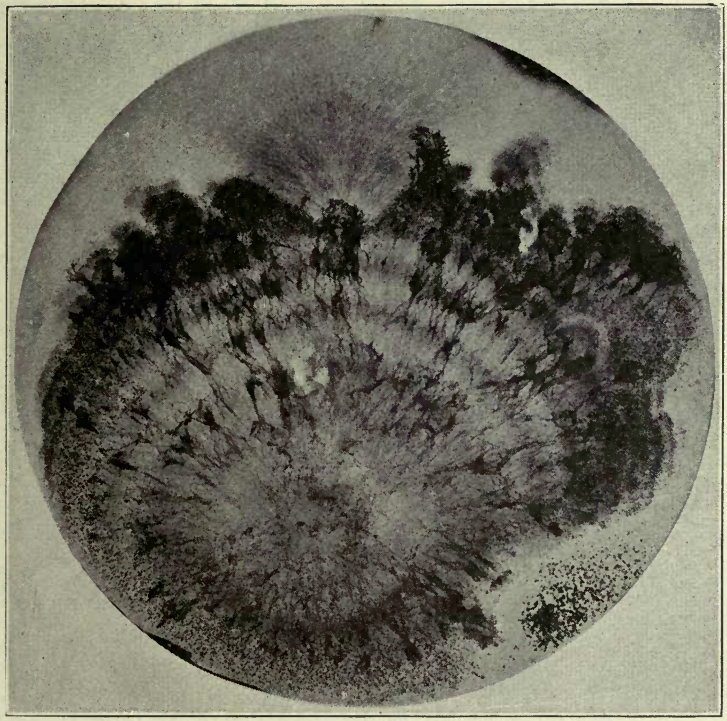
Diseased plant, showing numerous dead leaves and leaf-sheaths, also more than a dozen new shoots issuing from below the diseased portions. These shoots varied in height from a few millimeters to several centimeters.

PLATE IX



H. Nos. 1, 3, 4, 5, 20, and 22, growing on corn-meal agar.

Plate X



H. No. 36, showing very floccose mycelium.

PLATE XI

H. No. 3 (left) and No. 1 (right) as grown in Piorkowski-flask culture.

PLATE XI

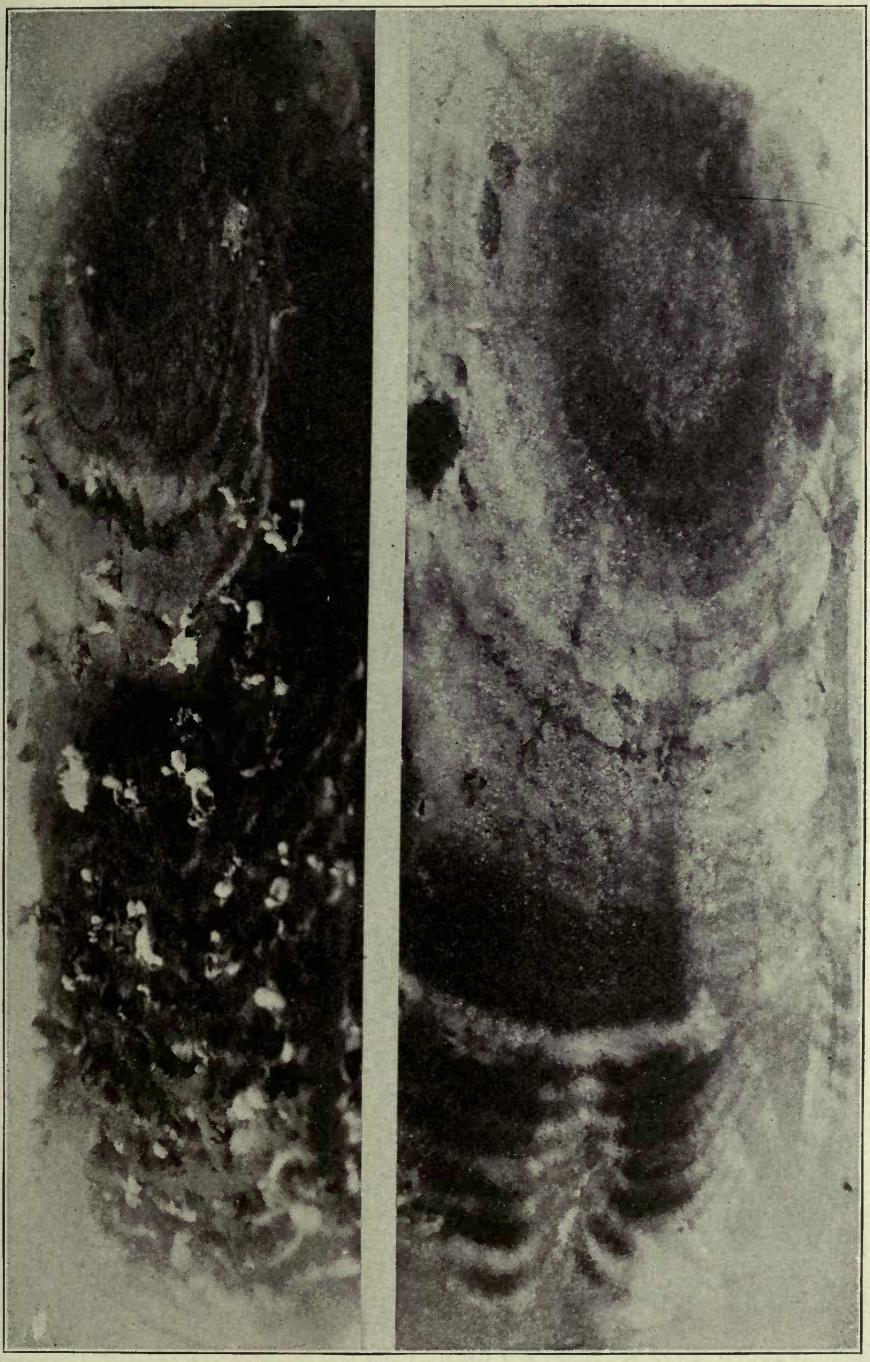


PLATE XII

H. No. 1 as grown in Kolle-flask culture.

PLATE XII

PLATE XIII

H. No. 3 as grown in Kolle-flask culture.

PLATE XIII

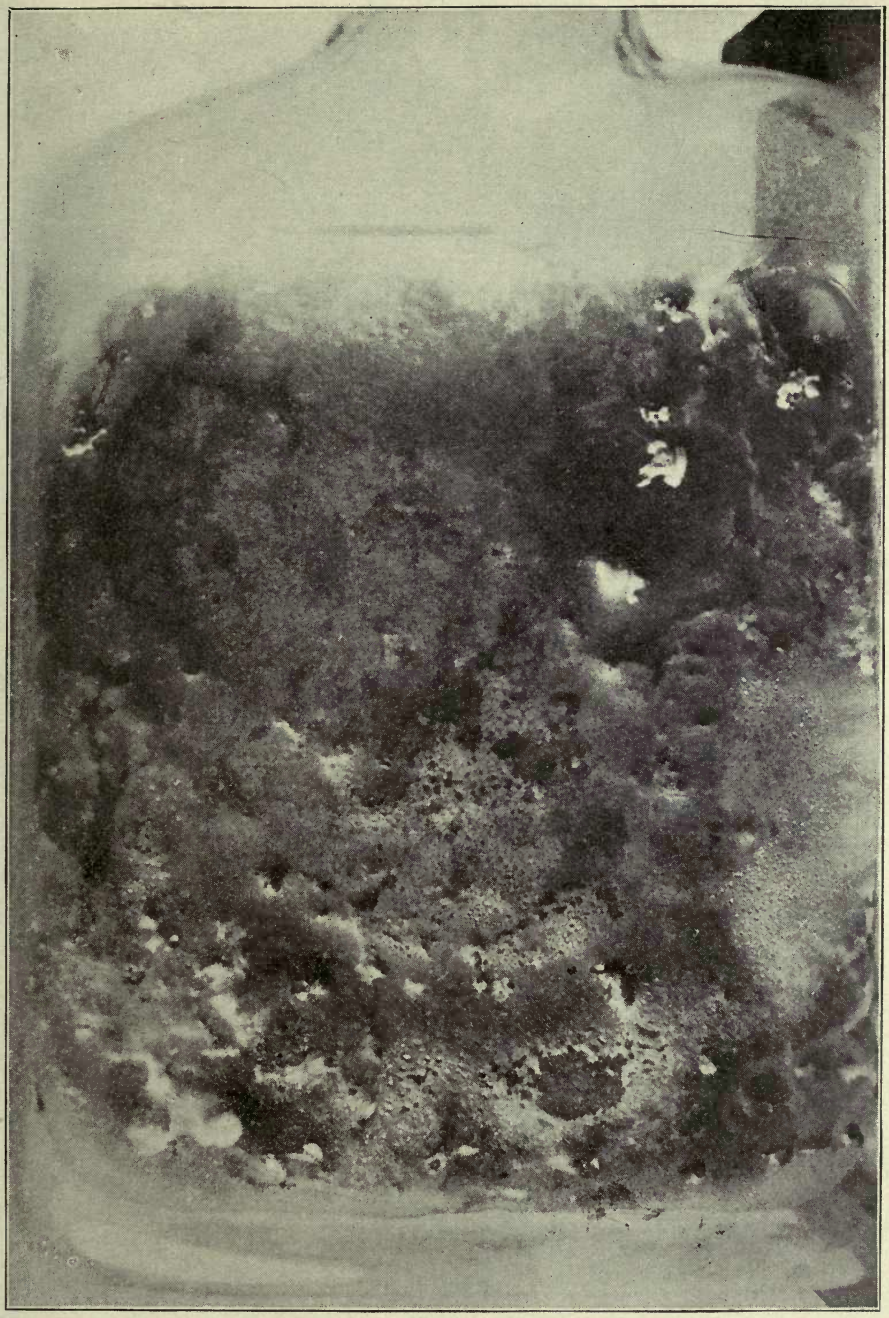


Plate XIV

Petri-dish cultures of H. No. 1 on different amounts of agar: 14, on 12 c.c.; 15, on 30 c.c.

PLATE XIV

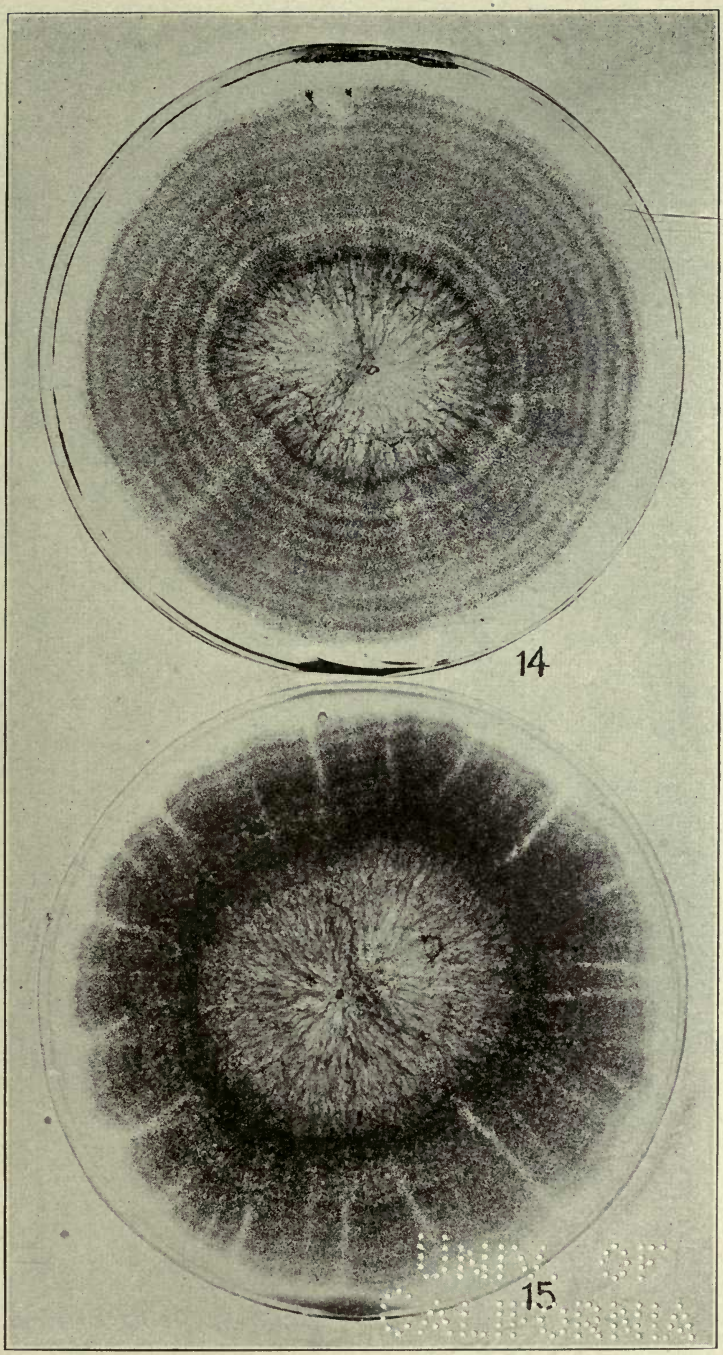
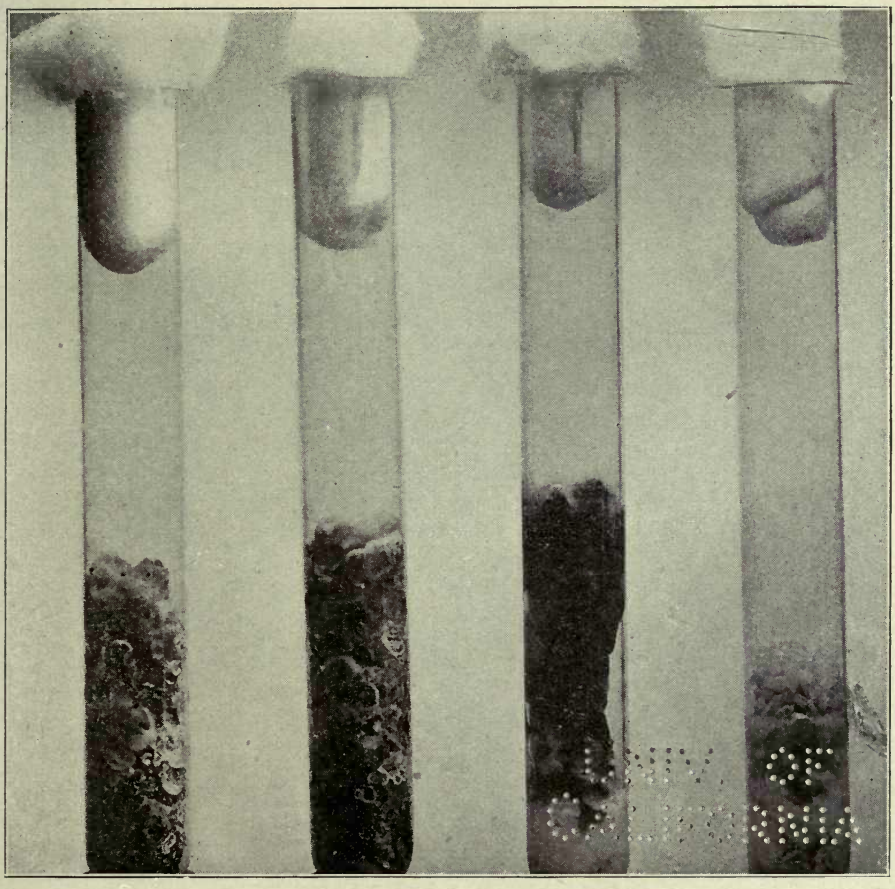
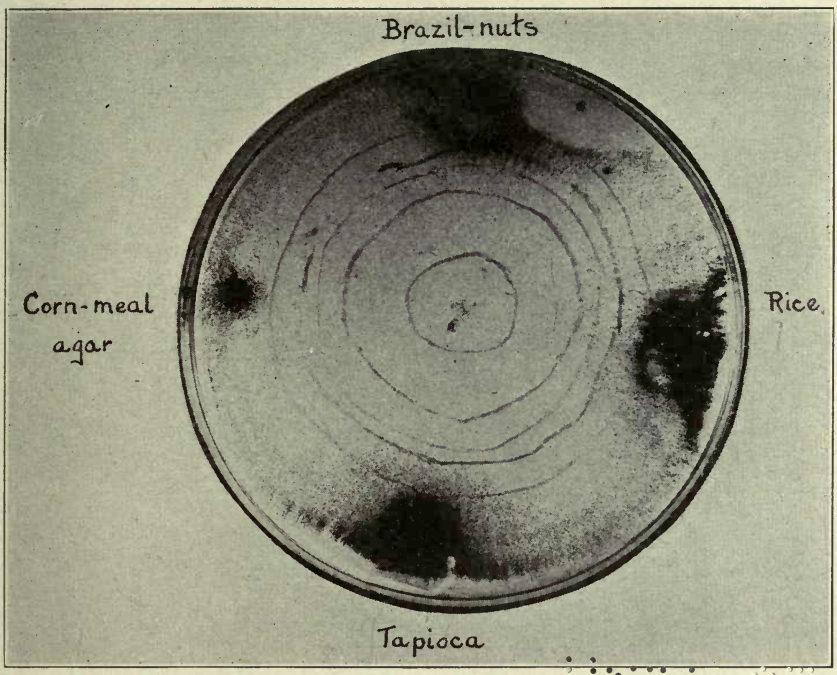


Plate XV



H. No. 1 growing in tubes of rice with different amounts of water. Note abundance of sclerotia in the drier tubes at the left.

PLATE XVI



H. No. 1 grown on washed agar with nutrients added as indicated—fragments of Brazil-nuts, rice, tapioca, and corn-meal agar. (Circles indicate approximate limits of growth at various periods.)

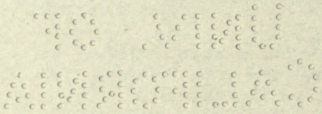
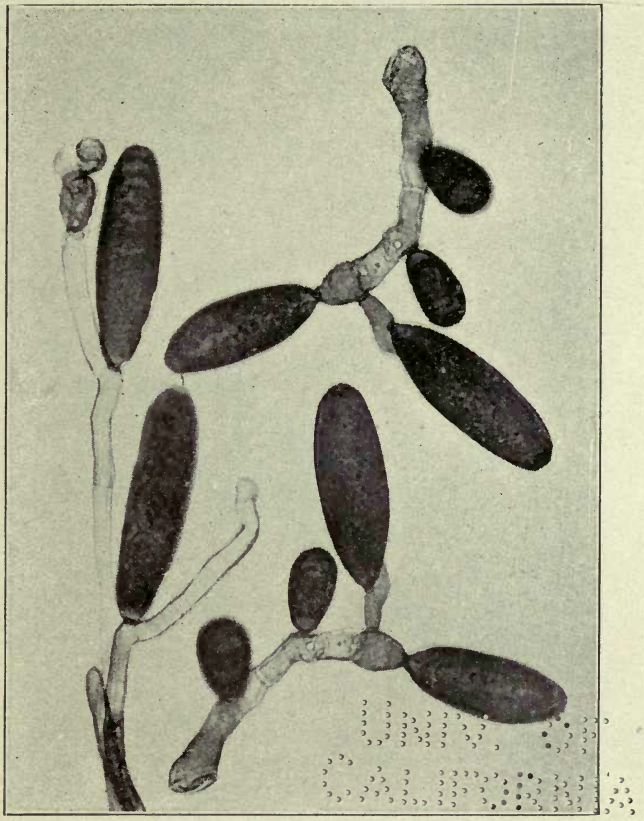
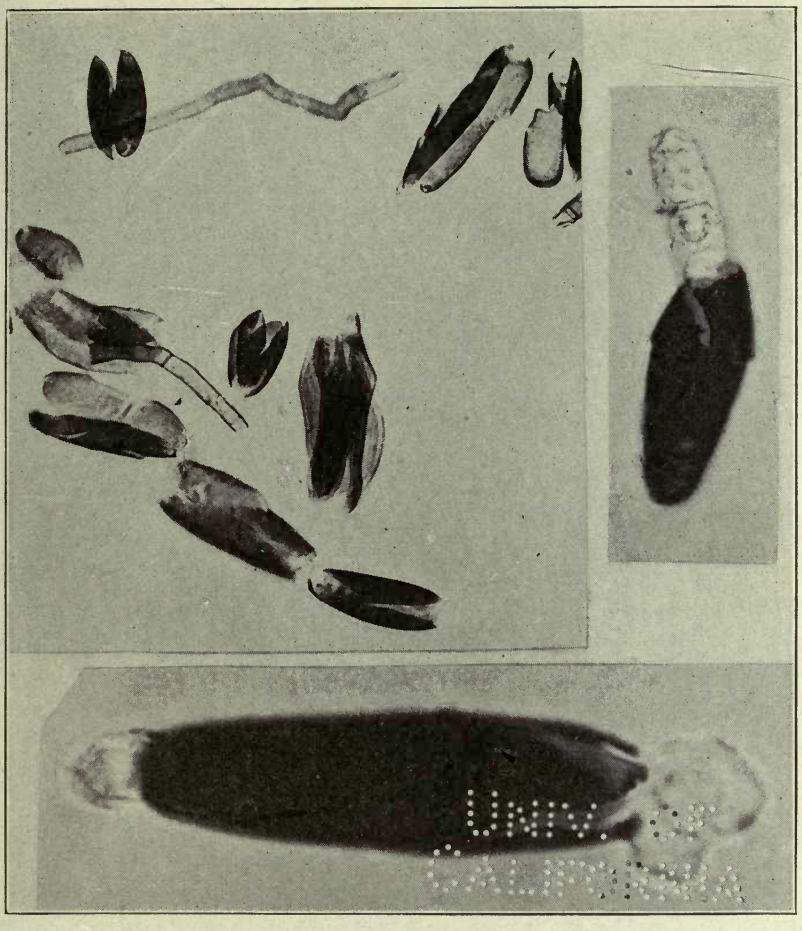


Plate XVII



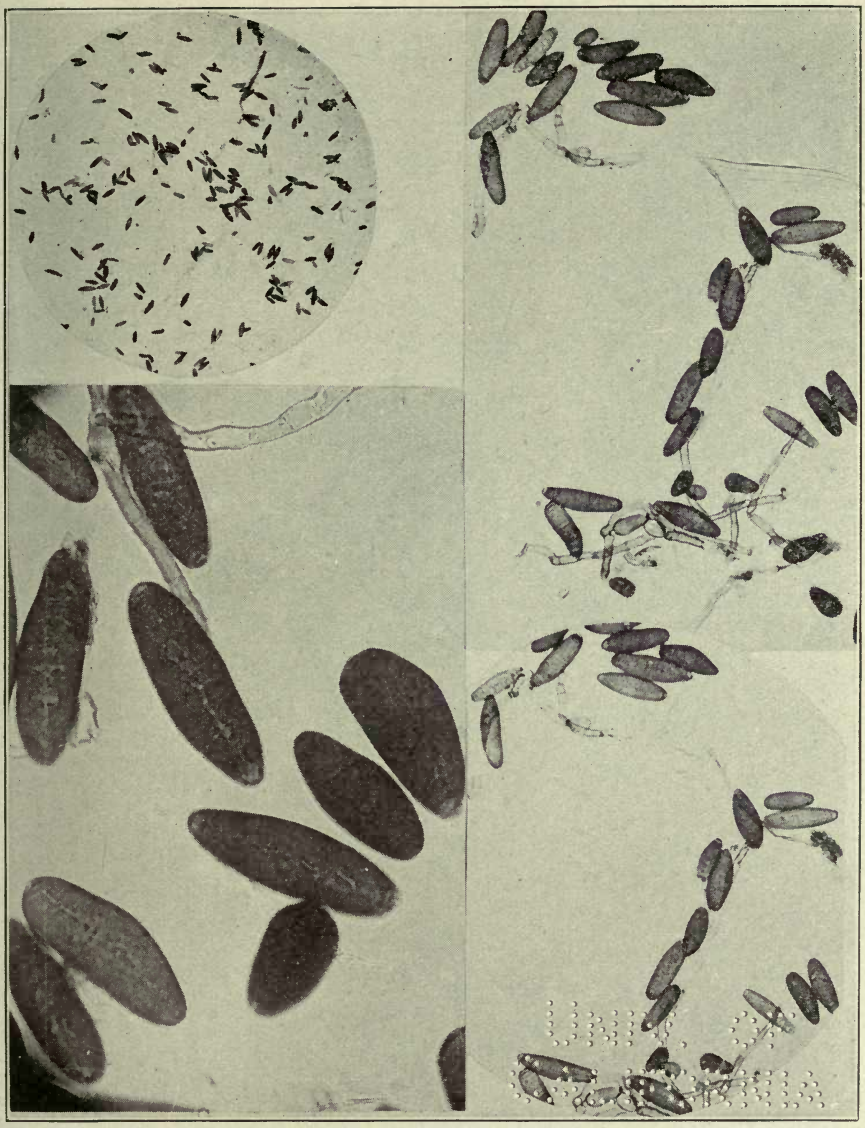
Photomicrographs of H. No. 1, showing attachment of conidia to conidophores.

Plate XVIII



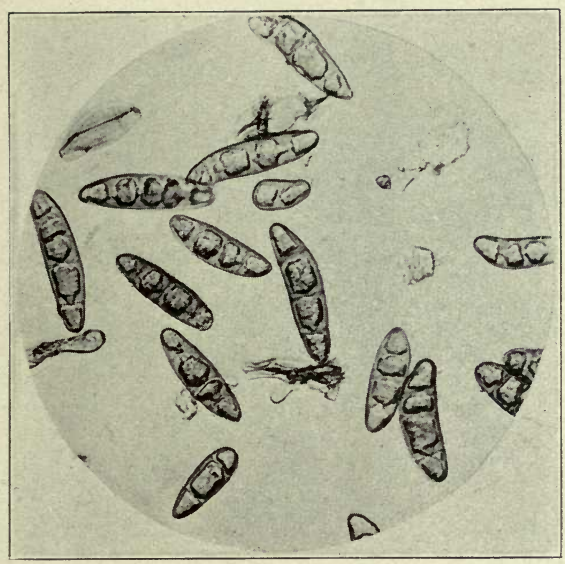
Photomicrographs of H. No. 1, showing the fragile nature of the outer brown] spore-wall and the gelatinous texture of the hyaline mass enclosed. (Three different magnifications.)

Plate XIX



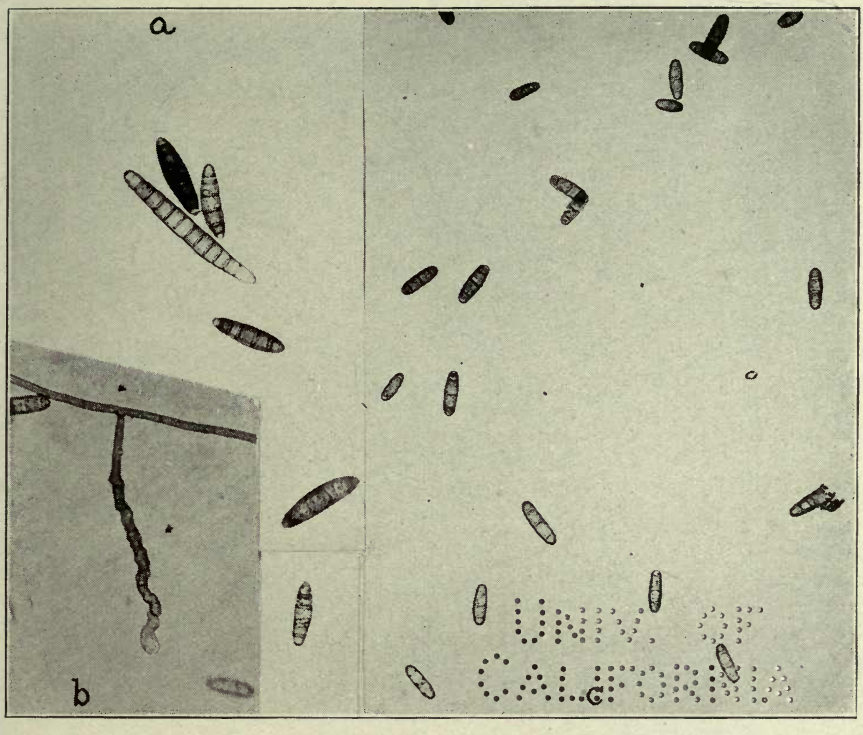
Photomicrographs of H. No. 1, showing conidia under different magnifications.

Plate XX

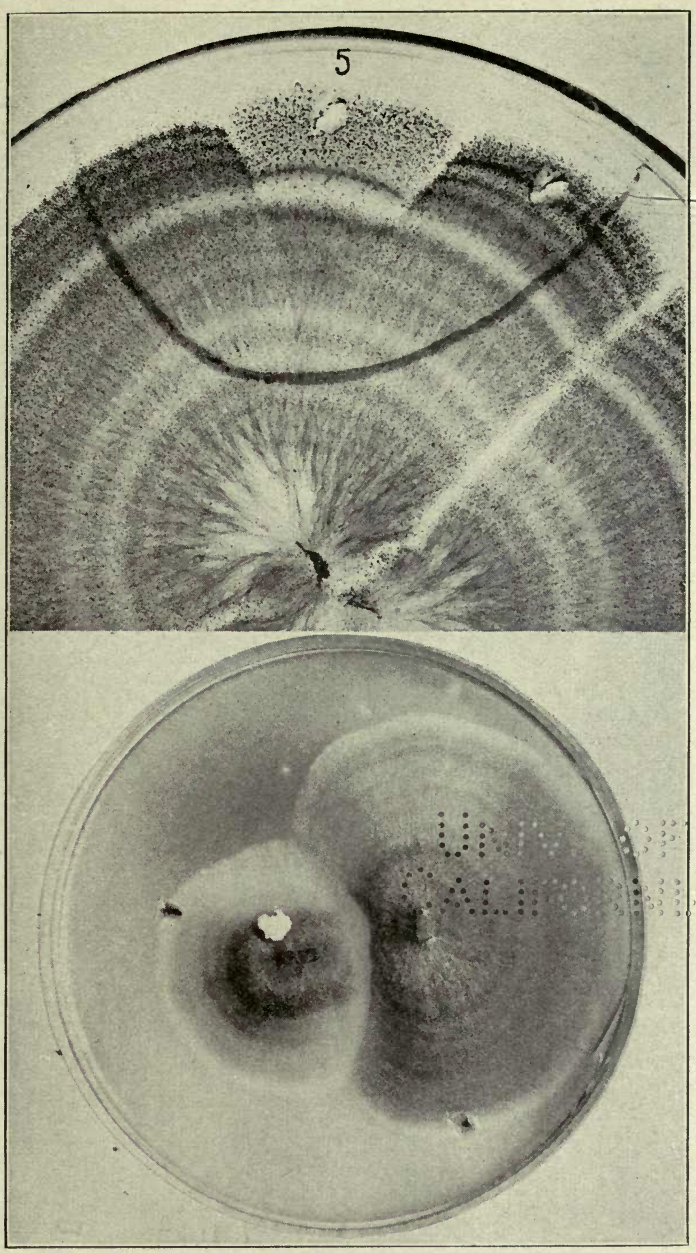


Photomicrograph of conidia of *H. ravenelii*.

Plate XXI

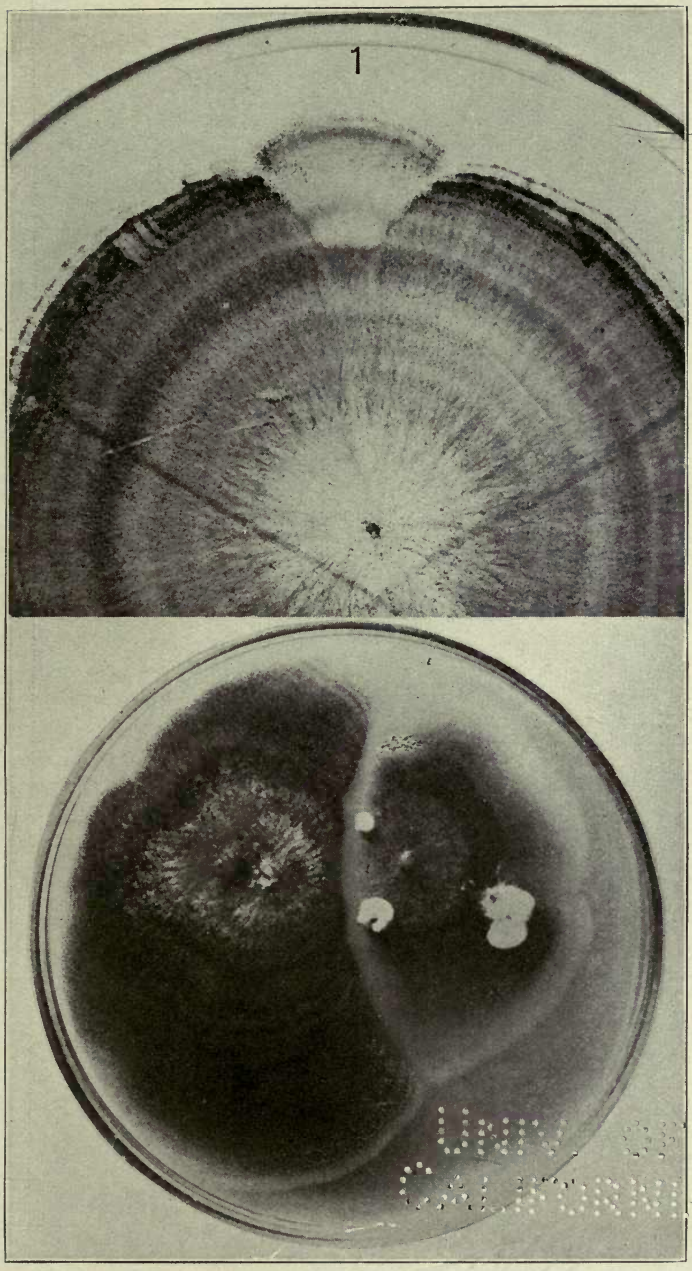


Conidia (*a*) of H. No. 36, showing variation in size and shape; *b* and *c*, conidia and a conidiophore of H. No. 39.

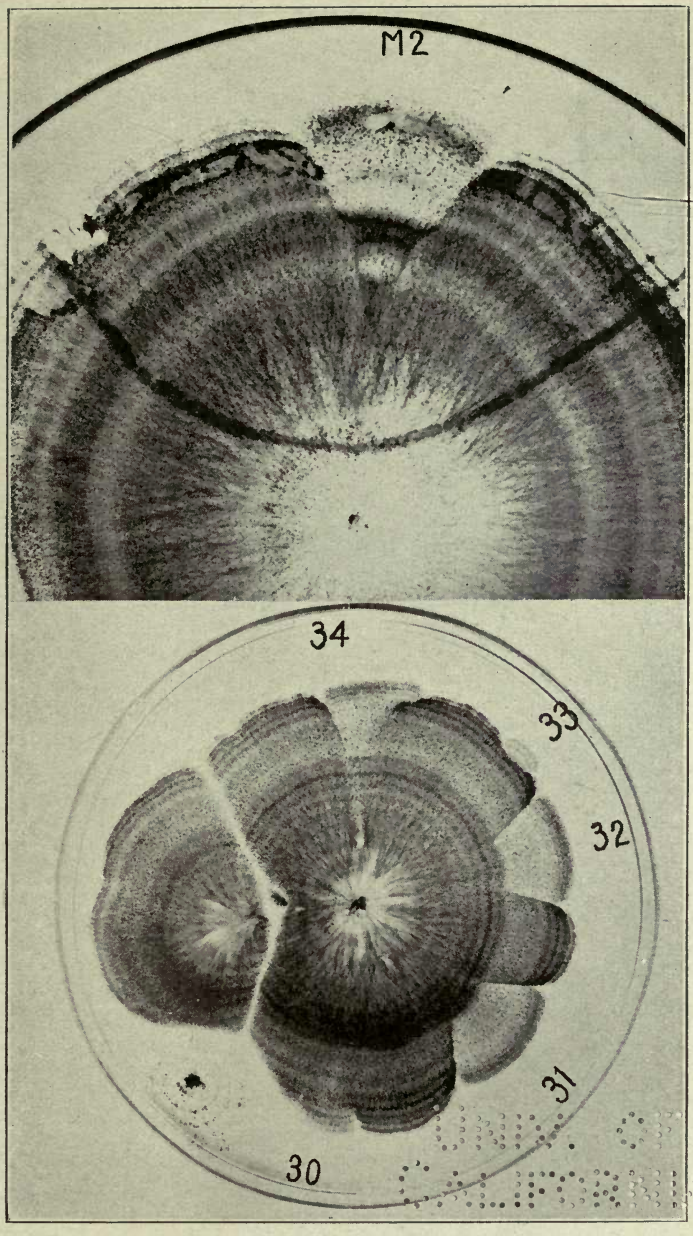


Two saltants: upper one showing origin of M5; lower one showing a white clump and slow growth.

PLATE XXIII



Two saltants: upper one showing origin of M1; lower one of slow growth and bearing clumps.



Upper figure showing origin of M2; lower figure showing origin of M30-M34.

PLATE XXV

Saltants growing with their respective originals.

PLATE XXV

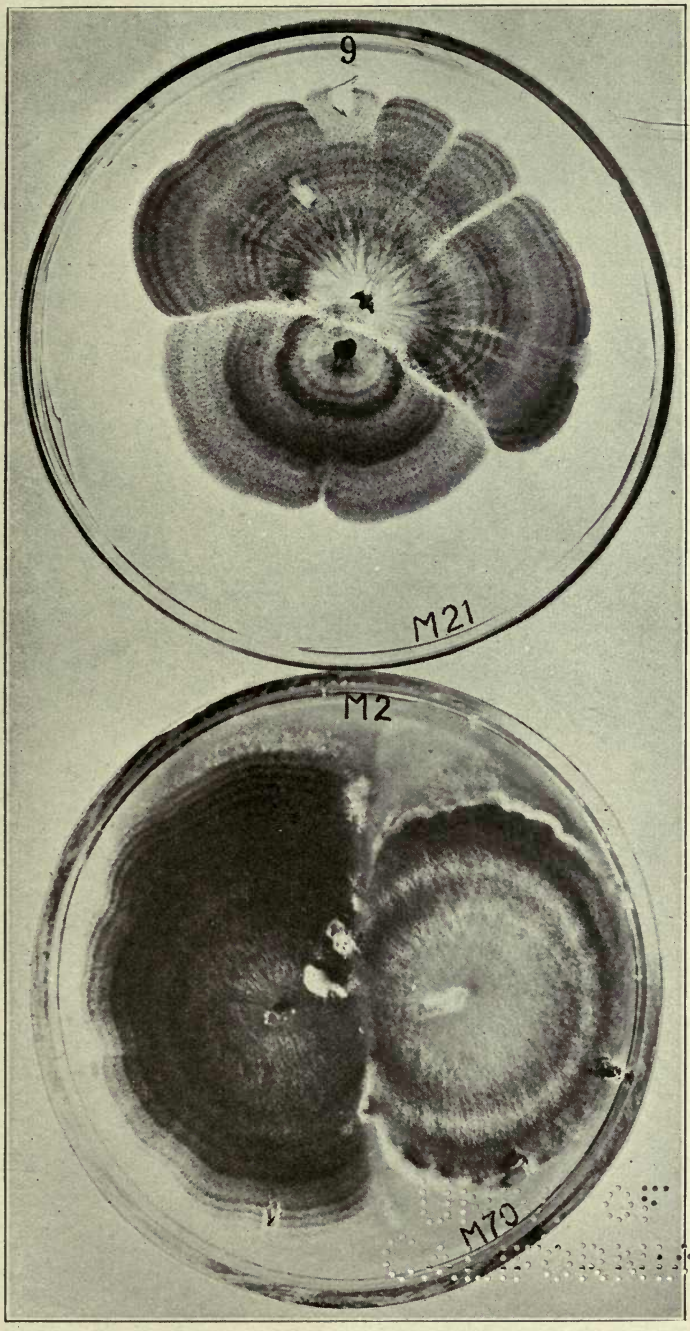


Plate XXVI

Photomicrographs (same scale) of conidia of several Helminthosporiums: *a*, H. No. 1; *b*, M6; *c*, M35; *d*, H. 20.

PLATE XXVI

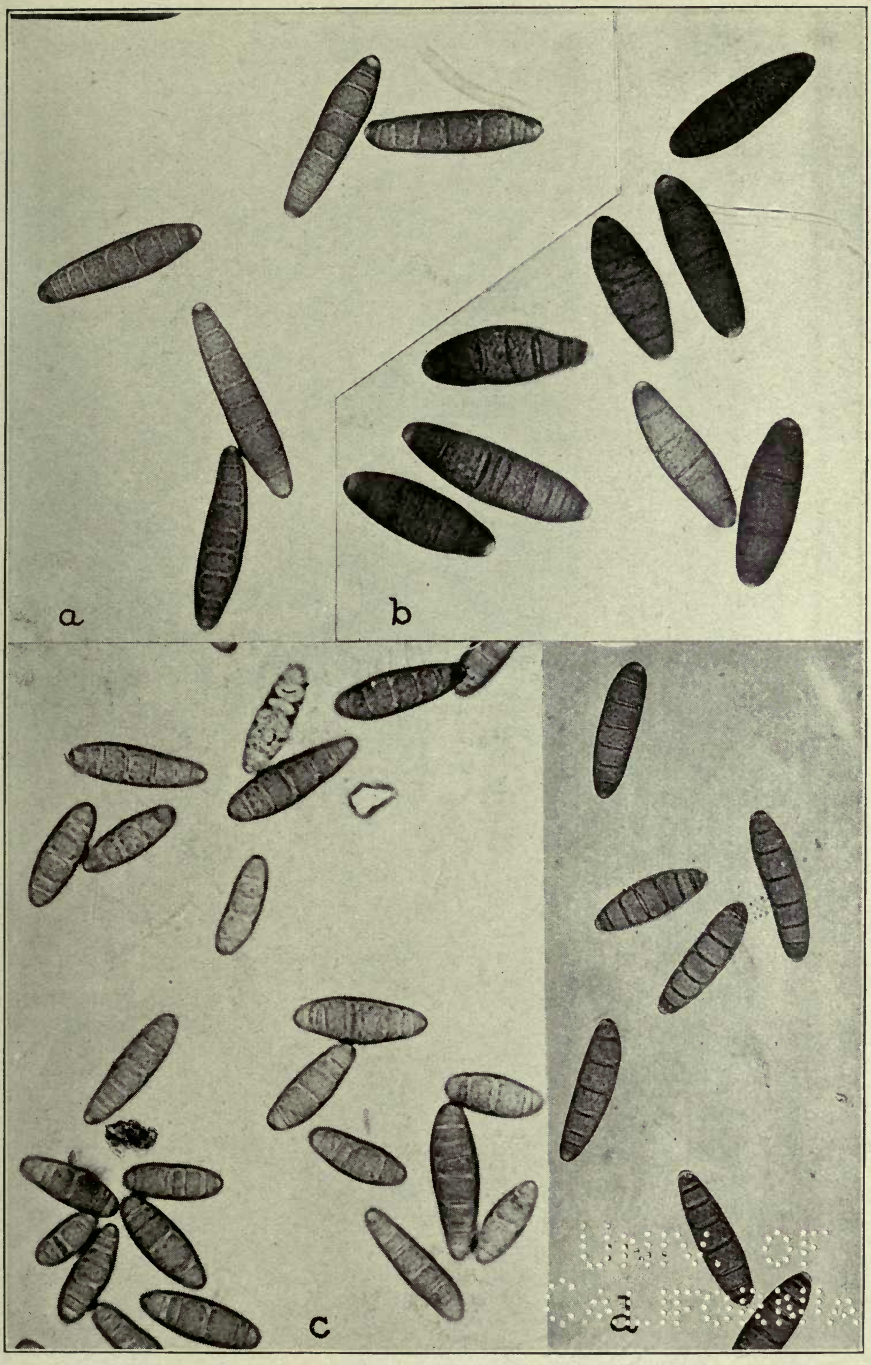
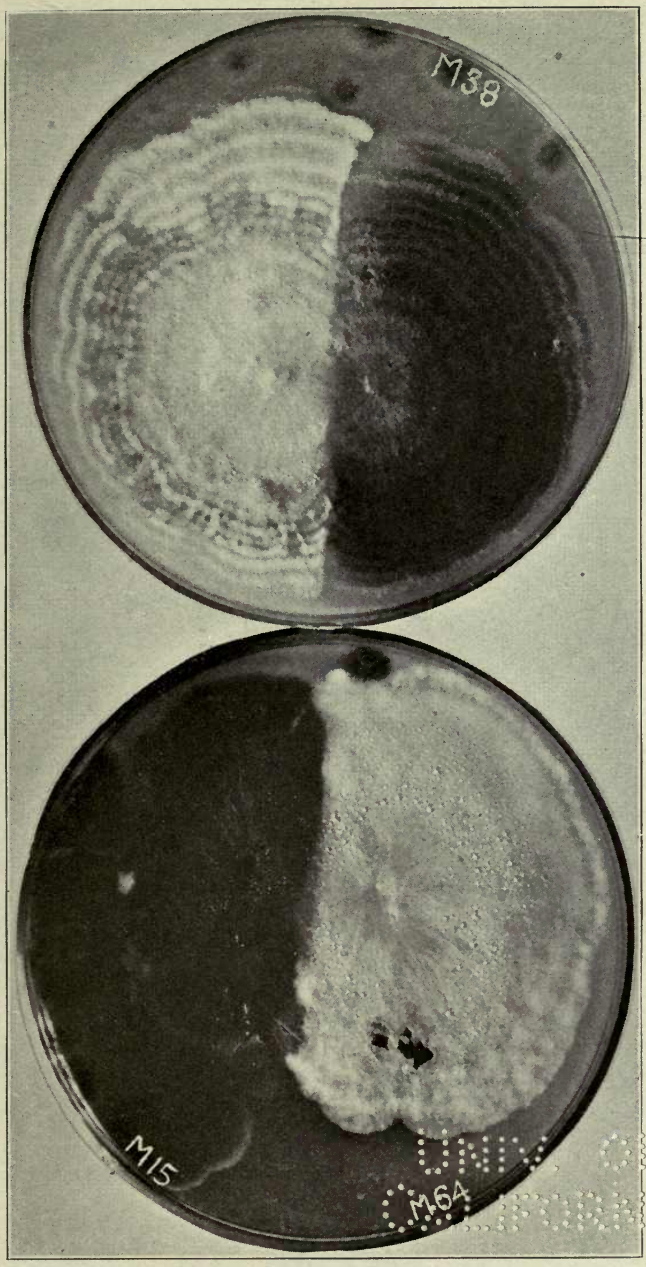
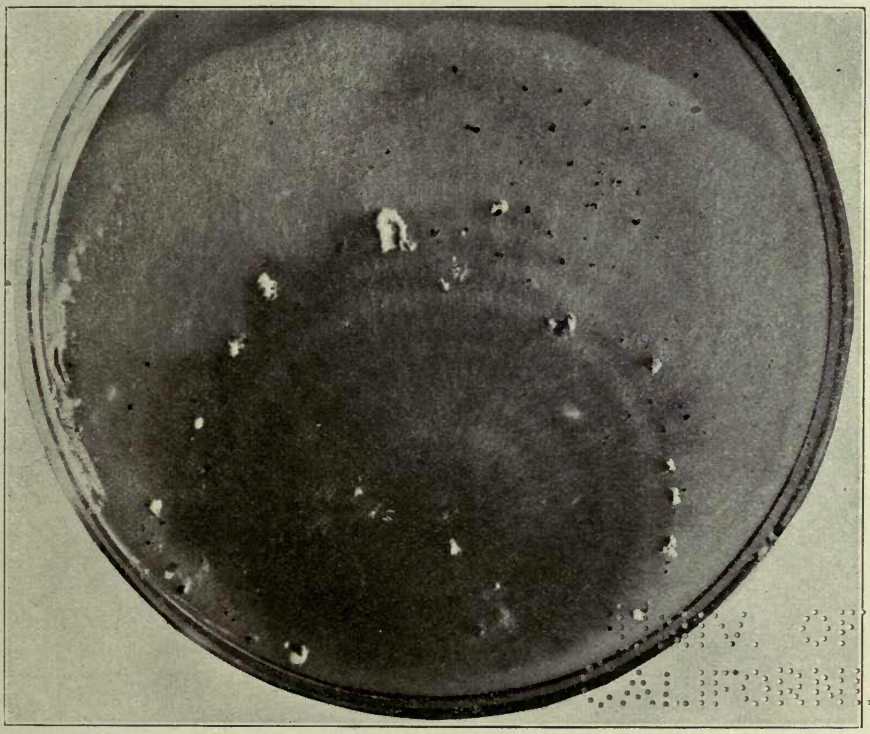


PLATE XXVII



Saltants growing with their respective originals.

Plate XXVIII



M34, characterized by abundance of sclerotia and white mycelial clumps, the latter a constant character of this saltant.

Plate XXIX

Above, H. No. 1 wounded by hot wire at points shown; below, H. No. 1, with H. No. 1 implanted at various distances within and without the colony.

PLATE XXIX

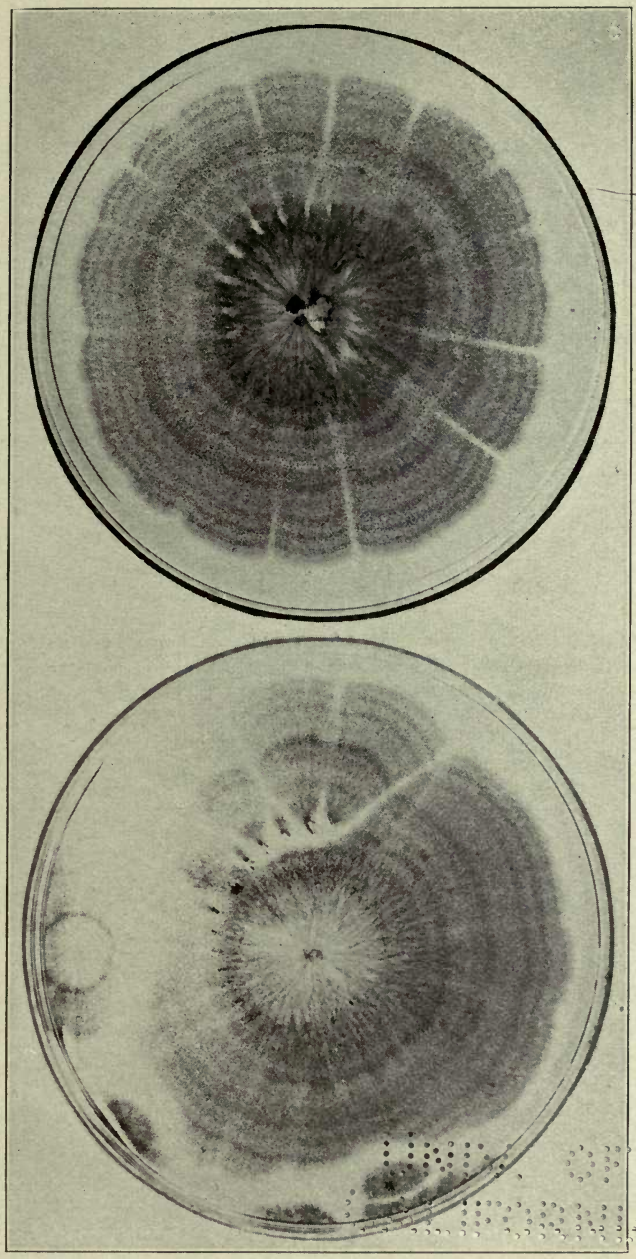


Plate XXX

Above, two implants of H. No. 1—one of them the origin of M70—in H. No. 1 colony, showing some white floccose aerial mycelium; below, M26, with origins of M53, M56, and M57.

PLATE XXX

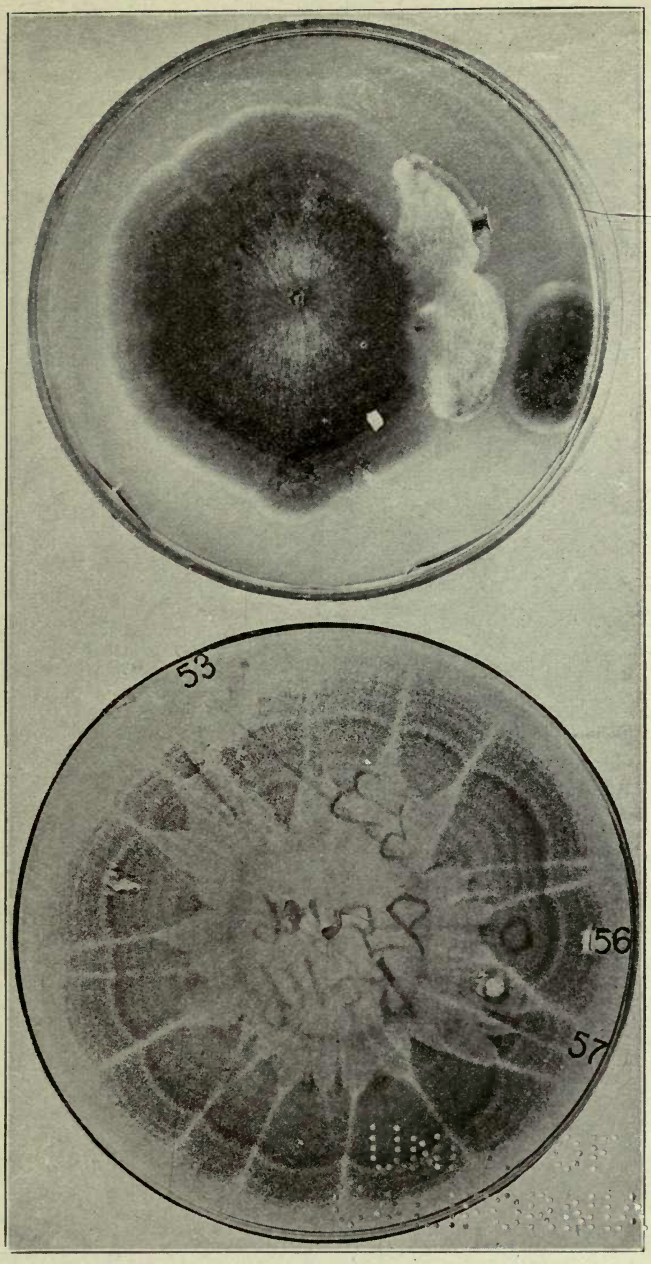


PLATE XXXI

M26-1 as it appeared on two separate plates.

PLATE XXXI

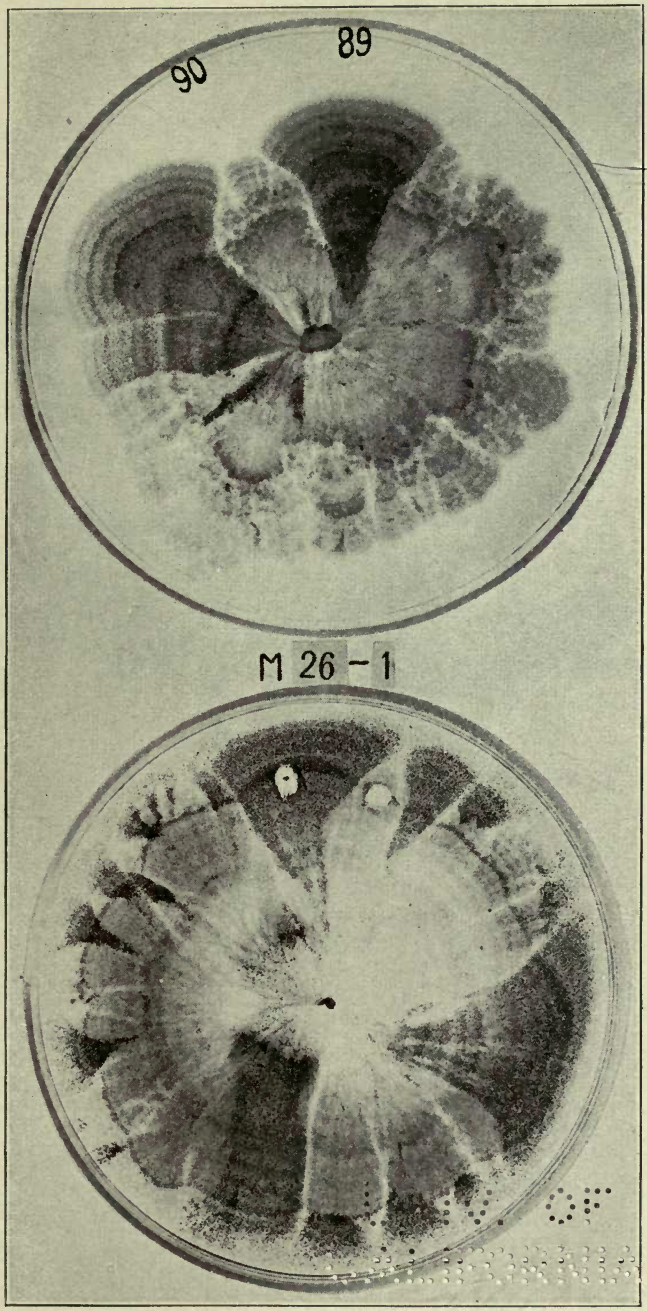


Plate XXXII

M125. Pale colonies, showing dark sectors which were apparently reversions to the original form.

PLATE XXXII

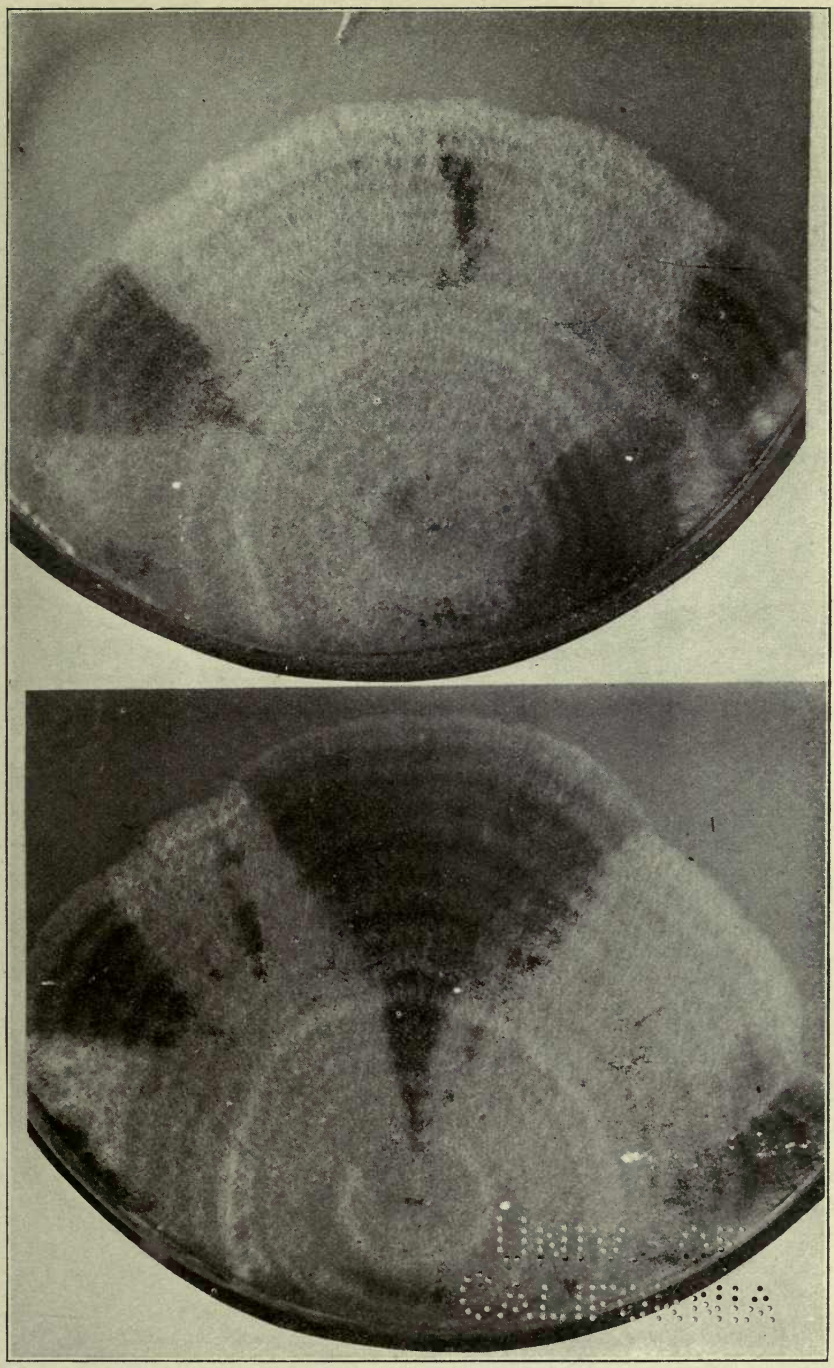


Plate XXXIII

Showing method of using rag doll in inoculations (*a*, *b*, *c*) and also (*d*) vial and paper cylinder for soil inoculation: *a*, the rag doll unrolled in sterile Petri-dish, and aseptic wheat seedlings in place, ready for inoculation; *b*, doll in place in tube and seedlings growing; *c*, showing development of root hairs in condition for inoculation below the doll; *d*, as stated above.

Plate XXXIII

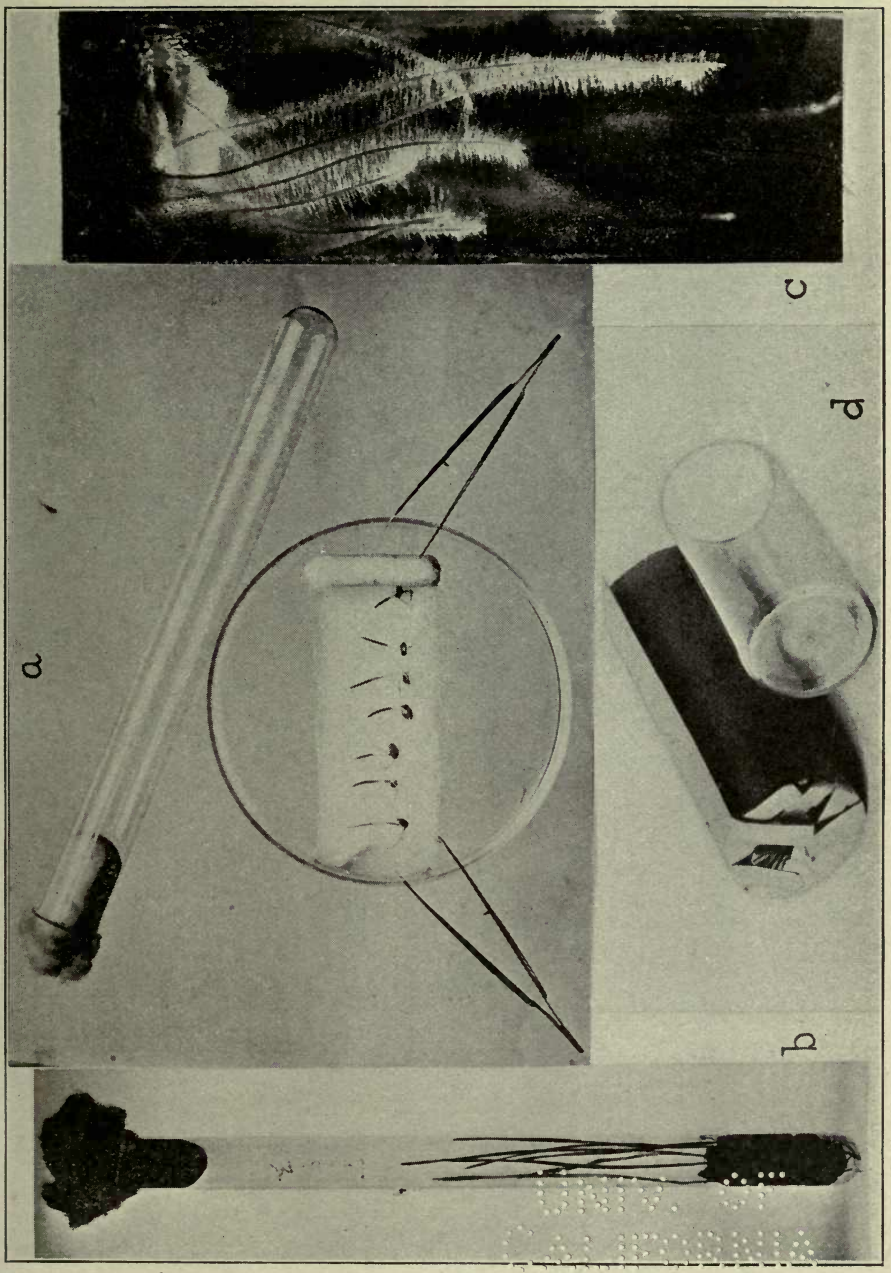


Plate XXXIV

Rag doll opened for examination 6 days after inoculation. All the seedlings show beginnings of foot-rot.